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8036 C*

Influence of Obstruction of the Bowel upon its Length and Weight.†

LOUIS SPERLING AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

No definite information is available as to the effect of obstruction upon the length and weight of the bowel. Because of expressed differences of opinion among clinicians relative to this matter, it was felt worth while to determine the influence of obstruction on bowel length and weight.

In 12 dogs segments of bowel one foot in length were marked off by placing silk ligatures on the mesenteric border of the bowel from the duodenum to the site of obstruction, the terminal ileum. The abdomen was then closed. After a period of time (Table I) the dogs were operated upon again and the length of each segment of bowel was remeasured.

The entire small intestines of 10 weighed normal dogs used in experimental demonstrations for physiology classes were obtained

* C represents a complete, P a preliminary manuscript.

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TABLE I.
Shortening of the Bowel in Simple Ileal Obstruction.

Dog	Days Obst.	Final Length of 12-inch Segments from Site of Obstruction, Ileum to Jejunum.								
1	4	8.75	9.00	7.50	9.00	9.50				
2	4	12.00	12.00	8.50	9.50	9.50	9.50			
3	4	9.50	9.00	8.00	7.00					
4	4	8.25	7.00	7.25	7.25	7.75				
5	4	7.25	6.75	7.00	7.00	8.25	8.50	8.25		
6	4	9.25	8.75	8.50	13.50	6.75	7.25			
7	2	9.50	10.00	10.00	11.50	10.50	12.00	11.00	9.00	
8	2	8.75	8.25	7.75	8.75	7.75	8.50	9.25	11.0	10.0
9	1	10.50	10.50	(Only two lower segments marked off)						
10	1	8.50	5.50	,,	,,	,,	,,	,,	,,	
11	3	10.00	8.00	,,	,,	,,	,,	,,	,,	
12	3	8.50	9.75	,,	,,	,,	,,	,,	,,	

immediately after death. The bowel was cut up into foot segments, the mesentery and fat were carefully dissected from the bowel wall, and the mucosa was cleaned by wiping with gauze. The segments were weighed to 0.1 gm., and segments of both jejunum and terminal ileum were desiccated in a drying oven at 100°C. for 4 days for a determination of the percentage of water content.

A similar procedure was carried out with the small bowel of 10 dogs with simple ileal obstruction of from 4 to 8 days' duration. In another group of 10 dogs, the bowel segments were marked off by tying a silk ligature on the mesentery at foot intervals from the duodenum to the site of obstruction, the terminal ileum. This was done at the time of operation immediately upon establishing the obstruction.

To determine more accurately the degree of circulatory stasis which occurs in obstruction and its effect on the weight of the bowel, the following experiment was carried out:

In 10 dogs under nembutal intravenous anesthesia a small segment of normal ileum was resected. Both proximal and distal ends were inverted with purse string sutures leaving a simple ileal obstruction and the abdomen was closed. At stated intervals they were operated upon again and another segment, the terminal portion of the obstructed bowel, was removed. The segments were cut free of mesenteric fat, cleaned by wiping with gauze, and reduced to a fine meaty pulp with scissors. Exactly 10 gm. of each specimen was extracted and its myo-hemoglobin determined by the method of Whipple, *i. e.*, by exposure to 0.15 N ammonium hydroxide (50 cc.) at ice box temperatures for 20 hours. The mixtures were then filtered through double layers of cheesecloth and the filtrates refiltered through fine filter paper. The resulting solutions of myo-hemoglobin were satu-

rated with carbon monoxide (illuminating gas) for 5 minutes. The solution of carbon monoxyl hemoglobin obtained from the normal bowel was compared colorimetrically with the solution prepared in exactly the same manner from the specimen obtained after a period of obstruction. The average of 5 readings was taken and values obtained as given in Table VI.

Results. In every case there was noted a definite shortening of the bowel, the length of a segment which before obstruction measured 12 inches decreasing by as much as one-third after obstruction. In only one instance was a segment of obstructed bowel longer than before obstruction. (Table I.)

According to several workers, the first response of the bowel of the guinea pig to distension is a contraction of the longitudinal muscle layers.[1] The shortening may be as much as 20 to 25% of its entire length. Van der Reis and Schembra[2] present observations to show that the functional length of the small bowel in man is only 2.2 to 2.7 meters or 6 to 15 feet in length. They contend that in the living person the bowel is much shorter than in the cadaver, when it usually measures about 22 feet.

It is here likewise shown that distension of the bowel accompanying mechanical obstruction in the dog also causes shortening. Hypertonicity of the obstructed bowel with marked contraction of the longitudinal muscular layer is probably largely responsible for this shortening.

The increase in weight of the ileum immediately above the site of obstruction was determined to be 114% when compared to the normal weight (Table III). When the factor of shortening of the bowel was eliminated, the true gain in weight was found to be 34% (Table IV). Water content comparison of normal and obstructed bowel showed an average increase of about 7% in the ileum above the site of obstruction and 4.7% in the jejunum remote from the obstruction (Table V). There is no dehydration of the bowel wall even though the systemic tissues are desiccated and the blood concentrated.

The weight per foot of the normal small intestine usually decreases from the duodenum to the terminal ileum. There seems to be a downward gradient, even in weight (Table II).

In simple ileal obstruction there is a reversal of the normal weight gradient, *i. e.*, the ileum above the site of obstruction weighs the

[1] Trendelenberg, P., *Arch. f. exp. Pharm. u. Path.*, 1916, **81**, 55; Crane, J. W., and Henderson, V. E., *Am. J. Physiol.*, 1924, **70**, 22.

[2] Van der Reis and Schembra, F. W., *Z. f. d. ges. Exp. Med.*, 1926, **52**, 74.

TABLE II.
Weight of Consecutive Feet of Normal Intestine from Ligament of Treitz to Terminal Ileum.

Dog	Weight kg.	Wt. of Consecutive 1-ft. Segments of Bowel, Jejunum to Ileum, in gm.
1	16	47—40—40—45—43—43—39
2	21	36—38—40—40—37—29—26
3	40	46—44—42—42—47—44—40—36
4	22	39—35—32—32—30—25—26—24—20—25—24
5	25	42—40—40—39—36—38—35—35—34
6	8	37—33—30—32—26—18
7	14	34—33—32—32—30—26—22—20—23—21
8	17	34—27—27—28—30—28
9	18	45—39—37—33—29—30—29
10	22	40—34—27—32—33—30—29
Aver. wt.	20	Aver. wt. of one foot of terminal ileum, 28.7 gm.

TABLE III.
Weight of Consecutive Feet of Obstructed Intestine from Ligament of Treitz to Site of Obstruction in Terminal Ileum.

Dog	Weight kg.	Days Obst.	Wt. of Consecutive 1-ft. Segments of Bowel, Jej. to Ileum, in gm.
1	18	7	42—45—51—52—62—70—60—85
2	19	8	41—34—36—36—42—40—41—41—49—68
3	19	6	52—45—40—54—50—43—62
4	19	5	46—44—36—50—55
5	23	6	39—43—48—42—48—62
6	17	4	43—52—50—39—46—55
7	16	6	42—46—46—54
8	18	6	39—39—43—56
9	12	7	30—34—49—52—48
10	15	5	56—56—58—72
11	19	7	29—21—20—19—23—26—30—51
Aver. wt.	17		Aver. wt. of terminal ileum per ft., 60.7 gm. Percent increase 114%

TABLE IV.
Weight Changes of Bowel in Simple Ileal Obstruction. Foot Segments Marked off at Time Obstruction Was Made.

Dog	Weight kg.	Days Obst.	Wt. of Consecutive 1-ft. Segments of Bowel, Jej. to Ileum, in gm.
1	14	4	40—32—31—26—29—52—41
2	13	4	21—22—23—30—34
3	14	4	41—45—44—42—57—58
4	18	4	27—25—35—33—37—35
5	8	4	23—16—18—19—35
6	17	4	31—31—35—50
7	10	4	24—20—21—21—27—27—27—39
8	11	2	22—22—20—19—22—23—22—26
9	13	2	25—24—22—25—23—23—24—26—30
10	14	3	21—20—22—22—29—37
Aver. wt.	13		Aver. wt. of terminal ileum per ft. .....38.5 gm. % increase 34%

TABLE V.

Water Content of Normal Bowel as Compared with Obstructed Bowel.

Dog	Normal Bowel Jejunum	Normal Bowel Ileum	Dog	Obstructed Bowel Jejunum	Obstructed Bowel Ileum
1	76.5%	72.6%	1	82.4%	85.1%
2	79.6	78.1	2	84.7	85.0
3	76.3	78.2	3	84.1	80.5
4	77.2	77.2	4	78.0	80.9
5	78.1	77.7	5	77.4	83.3
6	76.2	75.5	6	83.3	85.4
7	75.9	77.8	7	81.2	81.2
8	70.2	72.7	8	81.0	78.4
9	77.4	77.5	9	80.4	83.5
Aver.	76.3%	76.5%	Aver.	81.0%	83.5%
M.D.	±1.7	±1.73	M.D.	±2.39	±2.02

In simple ileal obstruction there is approximately 4.7% increase of water content of jejunum and 7% increase of water content of ileum.

most and there is a decrease in weight in a retrograde manner from the obstructed ileum to the duodenum. This is due to the fact that the segments of bowel immediately above the obstruction increase the most in weight, while those segments of the jejunum farthest away from the site of obstruction increase only slightly in weight (Table III).

The results indicated in Table VI represent relative values of the increase of hemoglobin content of obstructed bowel wall when compared with that of the normal bowel (same dog prior to obstruction). The solutions of myo-hemoglobin obtained from the normal bowel were very dilute, making colorimeter matching difficult. There was a wide range of individual variation, due, no doubt, to a variation in individual response to the conditions of obstruction. It was noted that values of more than 100% increase were the rule. A previous study indicated that this accumulation of blood in the bowel wall in simple obstruction is due to stasis in the vessels and is not interstitial hemorrhage in the gut wall.[3]

TABLE VI.

Comparative Increase of Hemoglobin of the Bowel Wall in Simple Obstruction.

Dog	Days Obst.	Comparative Increase Over Normal	Dog	Days Obst.	Comparative Increase Over Normal
1	1	5.34 x	6	3	1.20 x
2	1	2.76 "	7	4	10.65 "
3	2	3.22 "	8	4	7.17 "
4	2	2.80 "	9	4	2.75 "
5	3	1.40 "	10	4	4.54 "

[3] Carlson, H. A., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 421.

Summary and Conclusions. There is a marked shortening of the bowel in simple ileal obstruction (30 to 50%). The bowel wall above the site of simple ileal obstruction increases over 100% in weight. When the factor of shortening of the bowel is eliminated, the increase of weight is 34%. Edema of the bowel wall accounts for only 7% increase in weight. Next to shortening of the bowel the increase of blood in the bowel wall is most responsible for its great increase in weight.

8037 P

Comparison of Serum and Saline Extracts as Nutritive Media for Mammalian Lymph Node Cultures.

JOSEPH T. KING AND AUSTIN F. HENSCHEL. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

It has been reported by King (in press) that autogenous serum extracts of bone marrow promoted a more prompt and vigorous migration of lymphocytes, as compared with a saline extract of chick embryo, in cultures of mesenteric lymph node of the adult rabbit. It was also stated that the serum extract was definitely inferior to the saline extract as a coagulant for heparinized plasma.

Stenstrom and King[1] have reported the first study of a series on the effects of radiation of lymph node fragments. The next study is to be a more detailed consideration, the response of lymphocytes to radiation in such cultures. Since the response is prompt and since the characteristic activity of lymphocytes in such preparations is early, attempts were made to determine the medium of choice for promoting a prompt and vigorous lymphocyte migration.

The results of the comparison of the extracts referred to above encouraged further study to determine whether the desirable migration-promoting properties of serum marrow extract might be combined with the desirable coagulant properties of the saline embryo extract. To this end a study was made comparing saline and serum extracts of chick embryo.

In general technique followed was that described by King.[2] The mesenteric lymph node was removed promptly on stopping the cir-

[1] Stenstrom, Wilhelm, and King, Joseph T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 909.

[2] King, Joseph T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

culation. It was fragmented quickly in saline. The fragments were chosen in pairs, one of each pair being planted in each series. Attention was given to size, shape and color when choosing the fragments.

Blood was drawn into sterile heparin solution in such manner that 3 or 4 different concentrations of heparin were obtained. In this way a sensitive plasma was obtained.

Serum was obtained by drawing blood into a tube containing pieces of hard glass. It was defibrinated by moving the tube gently during clotting.

Extracts were made of 6-day chick embryos. Embryos were washed and extracted with serum or saline, 1 cc. of the medium being used per chick.

The cultures were incubated at 37.5°C. in Maximow slides. One drop of plasma and 3 drops of extract were mixed, the fragment oriented and the slide, with a vaseline ring inverted over the cover. The preparations were left until clotting had occurred when they were inverted and sealed with a vaseline-paraffin mixture. They were incubated as lying drops.

Observations were made at incubation, 3 hours, 1, 2, 3, and 4 days. Careful measurements were done on the original fragments, on the migration zone and the area covered by fibroblasts.

Observation at incubation showed that even at room temperature the lymphocytes were migrating quite uniformly in the serum series whereas there is rarely migration in the saline series. Long experience with saline extract cultures teaches that migration may occur in occasional instances. Here, however, the difference between the 2 series is striking and quite uniform. Not infrequently nearly all the cultures in the serum series will show quite a dense rim of lymphocytes around a large part of the periphery. In a single series part of the saline and part of the serum cultures were left at room temperature until the end of the 3-hour incubation period, at which time all the serum cultures showed rims to 15-20 eyepiece units (100 units = 1 mm.) while the saline series showed only scattered cells.

A general statement may be made concerning the appearance of the cultures for the 3-, 24-, and 48-hour observations. The serum series show earlier, more dense, more regular and more extensive migration as compared with the saline series.

The cells in the saline series tend to assume more extreme ameboid forms in spite of the fact that migration is not as vigorous.

Polyblastic proliferation starts earlier in the serum series and is much more dense.

No differences can be made out in the fibroblastic growth in the living preparations.

In general these differences parallel, at least in some degree, those already reported in the study on saline chick and serum marrow extracts and suggest that the characteristic difference noted there may be more a function of the extracting medium than the material extracted.

Concerning the coagulative properties of serum chick extracts, it may be stated that the result is much better than that found with serum marrow extracts. The clotting time is sufficiently short and the clots mechanically satisfactory.

The results of study of the fixed material will be presented in a final paper.

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8038 P

Insusceptibility of Young Puppies to Distemper Virus.

CHARLES A. SLANETZ. (Introduced by A. R. Dochez.)

From the Department of Animal Care, College of Physicians and Surgeons, Columbia University.

That newborn infants possess an effective resistance or immunity to certain communicable diseases may be considered a well established fact. This state of resistance endures for variable periods of time but in many instances sooner or later is replaced by susceptibility to the diseases in question, perhaps by an almost universal susceptibility as seems to be the case in measles. The nature of this immunity has been studied by many investigators who have remarked that it may be due to either active or passive immunization during intrauterine life by the passage of antigen or antibody through the placenta, to the transmission of antibodies from mother to child through the medium of colostrum or milk, or to some peculiar insusceptibility of young actively growing tissues to certain types of infection. McKhann and Chu[1] have recently demonstrated considerable concentrations of certain kinds of antibodies in salt solution extracts of human placentas. The subject has not been extensively studied in animals but it is known that antibodies essential to the well-being of the young are transmitted in colostrum as in the protection of newborn calves from colon bacillus infection. Fiadeiro[2] was able to actively immunize newborn pigs by inoculating them with living commercial hog cholera virus within three weeks after birth. No symptoms appeared. In the present study the susceptibility of newborn puppies to experimental infection with the virus of dog-distemper has been investigated.

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1 McKhann, F., and Chu, F. T., *J. Infect. Dis.*, 1933, **52**, 268.

2 Fiadeiro, J., *Rev. Med. vet., Lisboa*, 1934, **28**, 87.

Six litters of puppies were used in the experimental study. Five of the 6 mothers of these puppies after the birth of the animals were tested for immunity to distemper by an intradermal injection of 10 mg. of dried living distemper virus. All were found to be immune. Each litter was divided into 2 approximately equal groups, one to be inoculated and the other to serve as the uninoculated control. Thirteen puppies were injected intradermally at the age of one week with 10 mg. of living desiccated canine distemper virus (Lederle). Following these inoculations there was no rise in temperature nor were there any clinical signs of distemper. At the age of one month the same animals again received intradermally the same dose of distemper virus. Following this there was again neither a rise in temperature nor any apparent symptoms of distemper. At the end of 3 months the 2 groups of animals, consisting of the 13 previously inoculated and of 11 uninoculated controls received 10 mg. of living distemper virus intradermally. Following this procedure none of the previously inoculated animals displayed any signs of distemper. Of the 11 uninoculated controls, all developed distemper and 8 died. The remaining 3 had to be sacrificed after several weeks because of continuing bad condition. Because of heavy infestation with round worms 3 of the surviving inoculated animals were sacrificed 3 weeks after the last inoculation. At autopsy no evidence of infection with distemper was apparent. Of the original puppies inoculated with living virus a small number was kept under observation in the animal quarters for from one to 2 years. During this period they were exposed to contact infection with distemper but none contracted the disease.

In another group consisting of 4 puppies the effect of a single dose of living virus was tested. Two animals received 10 mg. of virus at the age of one week and 2 at the age of one month. At the age of 3 months these animals received 10 mg. of living virus intradermally. All 4 animals developed distemper but survived the disease.

During the course of these experiments the potency of the virus used was tested several times in ferrets. It was found that 10 mg. of the dried virus represented 10,000 lethal doses for ferrets.

An attempt was made to discover if invasion of the blood by distemper virus took place in the puppies originally inoculated with living virus. Two young animals were inoculated with virus intradermally and 2 days later 2 cc. of blood was withdrawn from the heart. This blood was injected into each of 2 ferrets subcutaneously. Neither of the ferrets developed signs of distemper. The

same ferrets proved susceptible 4 months later when they were injected with living canine distemper virus and died of distemper following the inoculation.

From these experiments one may draw the conclusions that inoculation of young puppies up to the age of one month with living distemper virus does not induce an attack of distemper. The nature of this insusceptibility is not known and may be due to inherited immunity, to immune substances taken in with the colostrum and milk or to some type of natural resistance. Five of the 6 mothers were immune to distemper at the time the litters were born. Administration by intradermal inoculation of living distemper virus to puppies during the first weeks of life in spite of producing no symptoms of disease seems to result in permanent immunity.

8039 C

Inactivation of Diphtheria Toxin in vivo and in vitro by Crystalline Vitamin C (Ascorbic Acid).*

CLAUS W. JUNGEBLUT AND RAYMUND L. ZWEMER.

From the Departments of Bacteriology and Anatomy, College of Physicians and Surgeons, Columbia University, New York.

For some years one of us (C.W.J.) has been actively interested in the study of natural resistance to infectious diseases, particularly in relation to poliomyelitis. This work has brought to light certain evidence which argues against the concept of universal latent immunization and favors acceptance of the view that the mechanism of natural resistance to this disease is purely physiological, depending essentially upon the function of the normal endocrine balance. While it is obviously very difficult in a problem as fundamental as this to determine precisely the particular rôle played by any one of the several glands of internal secretion, some experimental facts pointed very definitely to the significance of the anterior pituitary and the adrenal. It was also found that there existed a peculiar relationship between resistance to diphtheria and to poliomyelitis, suggesting the operation of a common protective factor in both diseases.

Meanwhile, the other author (R.L.Z.), on the basis of blood chemical and other studies, had been led to the belief that the ad-

* Under a grant from the Rockefeller Foundation.

renal cortex was involved in the course and regulation of metabolic and toxic demands on bodily resistance. This paper presents the first phase of a joint research program designed to correlate the mechanism of natural resistance in some infectious diseases, particularly diphtheria and poliomyelitis, with adrenal function.

Implication of the adrenal in diphtheria is suggested by a great many diverse observations. It is a well established fact that the adrenal glands of susceptible animals suffer heavily in this intoxication and that the natural insusceptibility of the rat to diphtheria and other bacterial toxins can be broken down by bilateral adrenalectomy.[1–6] It has also been shown that this artificially created lack of protection can be corrected again by supplying the operated animal with the deficient factor, *i. e.*, cortical extract.[7–10] More recently it has been possible to demonstrate that cortical extracts have the capacity of inactivating diphtheria toxin *in vitro*[11] and can raise the natural resistance of normal animals to excessive toxic[12] and other[13] demands.

Various biologically important substances found in the adrenal cortex, and possibly present in the extracts, were tested to determine their contribution to the end result. One of these, Vitamin C, gave such interesting effects that it was studied more intensively. The data obtained from approximately 100 guinea pigs is herewith presented.

The experimental work proceeded along 3 main lines of investigation. First, Vitamin C,† in varying quantities, was combined with

1 Scott, W. J. M., *J. Exp. Med.*, 1923, **38**, 543.

2 Lewis, J. T., *Am. J. Physiol.*, 1923, **64**, 506.

3 Belding, D., and Wyman, L. C., *Am. J. Physiol.*, 1926, **78**, 50.

4 Jaffe, H. L., *Am. J. Path.*, 1926, **2**, 41.

5 Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

6 Marmorston-Gottesman, J., Perla, D., and Vorzimer, J., *J. Exp. Med.*, 1930, **52**, 587.

7 Scott, W. J. M., and Bradford, W. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 428.

8 Perla, D., and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 475.

9 Hartman, F. A., and Scott, W. J. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 478.

10 Perla, D., and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 650.

11 Jungeblut, C. W., Meyer, K., and Engle, E. T., *J. Exp. Med.*, 1934, **27**, 43.

12 Zwemer, R. L., and Jungeblut, C. W., (in preparation).

13 Wolfram, J., and Zwemer, R. L., *J. Exp. Med.*, 1935, **61**, 9.

† We are grateful to Merck and Company for placing at our disposal a generous supply of Cebione, their natural crystalline Vitamin C preparation.

diphtheria toxin (2 m.l.d.) and the mixtures after standing one-half hour at room temperature were injected subcutaneously into guinea pigs. Second, guinea pigs were injected by the subcutaneous route simultaneously but in separate locations with Vitamin C and diphtheria toxin (2 m.l.d.). Third, guinea pigs received Vitamin C for several days so as to ensure a high storage of this substance, and were subjected subsequently to a series of intracutaneous injections of small doses of diphtheria toxin. All the guinea pigs used in Series I and II and their controls weighed from 230 to 280 gm. In Series III larger albino animals of about 350 gm. weight were employed. The diphtheria toxin used throughout this work represented one batch of stabilized filtrate, containing from 400 to 450 m.l.d. per 1 cc., so that 1 cc. of a 1:200 dilution was equivalent to at least 2 minimal lethal doses.

Controls. Each test was accompanied by one or more control animals receiving the toxin alone or in combination with 1 cc. of saline. All in all, 22 control guinea pigs were injected with 2 m.l.d. as calculated above. Without exception these animals died within from 24 to 72 hours with typical symptoms of diphtheria intoxication (Table I).

TABLE I.
Injection of Normal Guinea Pigs with 2 m.l.d. of Diphtheria Toxin.

No. of G. Pigs	Dose of Toxin	Died within 24 hr.	48 hr.	72 hr.
22	2 m.l.d.	1	13	8

Series I. A total of 37 guinea pigs were used in the first series. While the dose of toxin was kept constant (2 m.l.d.), the amount of Vitamin C varied from 0.05 mg. to as much as 100 mg. In order to guard against the possibility of nonspecific acid destruction of the toxin in these tests in which toxin and ascorbic acid were brought into direct contact, the solution of Vitamin C was adjusted to pH 6.6 to 6.8 immediately before combining it with the toxin. This pH had no deleterious effect on the potency of the toxin as shown by control experiments in which animals receiving a mixture of 2 m.l.d. of toxin and lactic acid of the same pH died as promptly as the controls injected with toxin and saline. This observation, moreover, is in harmony with Walbum's conclusions on the degree of acid-resistance of diphtheria toxin.[14] As may be seen from Table II there is a very definite range within which inactivation of diphtheria toxin may regularly be obtained by Vitamin C. The optimal quan-

[14] Walbum, L. E., *Biochem. Z.*, 1922, **130**, 25.

TABLE II.
Inactivation of Diphtheria Toxin by Vitamin C *in vitro*.

No. of G. Pigs	Dose of Vitamin C	Dose of Toxin	Died within 96 hr.	Died between 5 and 9 days	Survived
	mg.	m.l.d.			
5	100	2	3		2
4	50	2	3	1 (7d.)	
4	10	2	1	2 (5d., 6d.)	1
4	5	2			4
4	1	2			4
4	0.5	2			4
4	0.2	2		1 (7d.)	3
4	0.1	2	2	2 (6d., 9d.)	
4	0.05	2	4		

tities seem to lie between 0.5 mg. and 5 mg., larger or smaller doses of Vitamin C failing to protect with the same consistency. The failure of excessive doses to inactivate is of special interest since it seems to give evidence against a purely physical or chemical destruction of the toxin by this substance.

Series II. The second series comprises a total of 14 guinea pigs which were injected with 2 m.l.d. of diphtheria toxin and at the same time received a separate subcutaneous injection of different amounts of Vitamin C in acid solution, varying from 1 mg. to 200 mg. (Table III). These experiments show that under the

TABLE III.
Inactivation of Diphtheria Toxin by Vitamin C *in vivo*.

No. of G. Pigs	Dose of Vitamin C	Dose of Toxin	Died within 96 hr.	Died between 5 and 10 days	Survived
	mg.	m.l.d.			
2	200	2	1		1
2	100	2	1		1
2	50	2	1		1
2	25	2	1		1
2	10	2	1	1	
2	5	2	1		1
2	1	2	2		

conditions of the test, approximately half of the animals survived which had received between 5 mg. and 200 mg. of Vitamin C. In contrast to the results obtained in the mixture tests there was no evidence of a relation between the dose of Vitamin C injected and the outcome of the experiment, except that with the smallest dose given both animals died. It is noteworthy that no greater regularity could be obtained in some additional tests in which the dose of Vitamin C was repeated the following day.

Some of the guinea pigs surviving in this and the first series

showed slight ulcerations at the site of injection and in some cases peripheral paralysis after an interval of approximately 2 weeks.

Series III. The above work made it clear that although inactivation of diphtheria toxin by Vitamin C is obtainable *in vivo,* yet our results lacked the precision observed with *in vitro* tests. It was therefore thought advisable to investigate the possibility of inducing an enhanced resistance against minute doses of diphtheria toxin in guinea pigs which had been allowed to store Vitamin C for 6 days before intracutaneous injection of the toxin.

A group of 13 guinea pigs were prepared by daily injection of various amounts of Vitamin C in either acid or partly neutralized solution. A modified silver nitrate treatment[15] of sections from the adrenals of some of the prepared animals demonstrated an increased reduction by ascorbic acid. The same method had previously shown

TABLE IV.
Effect of Vitamin C Storage on the Reaction of Guinea Pigs to the Intracutaneous Injection of Small Doses of Diphtheria Toxin.

Guinea Pig No.	Daily dose of Vitamin C for 6 days mg.	Reaction after 24 hr. 1/50 m.l.d.	1/100 m.l.d.	1/200 m.l.d.	1/500 m.l.d.	Reaction after 72 hr. 1/50 m.l.d.	1/100 m.l.d.	1/200 m.l.d.	1/500 m.l.d.
		Tests on Vitamin C Stored Guinea Pigs.							
1	100	0	0	0	0	2	2	1	ø
2	100	2	2	1	1	2	2	1	1
3	100	0	0	0	0	2	2	2	ø
4	100	2	2	0	0	5	4	ø	ø
5	100	2	2	2	1	4	2	1	1
6	50	1	1	1	0	0	0	0	0
7	50	2	2	1	1	2	2	1	1
8	10	3	3	2	2	5	4	2	ø
9	10	3	2	2	2	2	2	ø	ø
10	10	3	2	2	1	5	ø	ø	ø
11	1	3	2	1	1	4	ø	ø	ø
12	1	2	2	1	1	2	2	ø	ø
13	1	3	3	3	2	5	2	1	1
		Tests on Control Guinea Pigs.							
14	—	3	3	3	2	5	4	2	2
15	—	2	2	2	2	5	4	3	3
16	—	3	3	2	2	4	4	4	2
17	—	3	3	2	2	4	4	3	2
18	—	2	2	2	1	4	3	ø	ø
19	—	3	3	2	2	5	3	3	2
20	—	2	2	2	1	5	5	4	3
21	—	3	3	3	2	5	3	3	ø
22	—	3	3	3	2	5	4	3	3
23	—	3	2	2	1	5	4	3	3
24	—	3	3	2	2	5	4	2	1
25	—	3	3	2	1	4	4	2	1
26	—	3	3	2	2	5	5	5	4

Key explained in the text.

[15] Moore, T., and Ray, S. N., *Nature,* 1932, **130**, 997.

a diminution of Vitamin C in this organ in the course of diphtheritic and poliomyelitic infection. The animals were shaved on the sides and 4 doses of diphtheria toxin, *i. e.*, 1/50, 1/100, 1/200 and 1/500 m.l.d. contained in a volume of 0.1 cc., were injected intracutaneously, two on each side. Readings were recorded every 24 hours for 5 days. For tabulation we have selected only the 24-hour and 72-hour results as being most significant and characteristic of the trend of events. Every experiment was controlled by the inclusion of one or 2 normal animals, the latter numbering 13 in all. To secure maximal accuracy in comparing the results in both groups of guinea pigs, the size and degree of redness as well as the intensity of necrosis for each individual animal are recorded in Table IV according to the following key: 0 = negative; 1 = v. sl. red (1 x 1 cm.); 2 = sl. red (1½ x 1½ cm.); 3 = red (2 x 2 cm.); 4 = mod. necrosis; 5 = heavy necrosis; ∅ = faded.

It appears from Table IV that the storage of Vitamin C had a very pronounced effect either in inhibiting or completely suppressing the local reaction to diphtheria toxin. In the controls, the average reading after the first 24 hours was 3322, whereas most of the prepared animals had much lighter reactions. In 2 instances there was no trace of any reaction. At the end of 72 hours the average control reading was 5432 while the majority of the prepared animals had no necrosis even with the largest amount of toxin injected and exhibited much lighter reactions with the smaller doses. In keeping with the results of Series II, individual differences in response of the prepared animals sometimes seemed more important than the experimental variation in the amount of Vitamin C injected. As a rule, however, the larger doses apparently afforded better protection.

We conclude from these data that Vitamin C inactivates diphtheria toxin and helps to protect guinea pigs against the fatal outcome of diphtheria intoxication. Also that guinea pigs, injected with suitable amounts of this substance are rendered temporarily negative or definitely less sensitive to small doses of the toxin as determined in intracutaneous tests. The experiments therefore indicate an important part played by Vitamin C in the mechanism of natural resistance to diphtheria toxin.

After completion of this work it was found that a paper reporting similar results with Vitamin C and diphtheria toxin had been presented at the meeting of September 24, 1934, of the French Academy of Sciences, by E. Harde.[16]

[16] Harde, E., *C. R. Ac. Sc.*, 1934, **199**, 618.

8040 C

Effect of Nembutal upon Serum Cholesterol of Dogs.*

EMILY H. BIDWELL, FREDERICK H. SHILLITO, AND KENNETH B. TURNER.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

In certain operative procedures upon dogs, nembutal was used as the anesthetic. It was necessary to know in these animals whether nembutal *per se* influenced the level of cholesterol in the blood. There are numerous references in the literature to the effects of various anesthetics, particularly ether and chloroform, upon the blood lipids. We were, however, unable to find any report concerning the action of nembutal upon the blood cholesterol.

Because of the absence of this information about an anesthetic widely used, particularly in experimental work, we were prompted to make this brief report.

A fresh 10% solution of nembutal (Abbott) in normal saline was injected intraperitoneally in 7 dogs. Approximately 40 mg. per kg. body weight were sufficient to produce deep narcosis lasting several hours. A sample of blood was obtained before the nembutal was

TABLE I.

Dog No.	Hours after nembutal	Serum cholesterol mg./100 cc.
169	0	184
	5	179
304	0	149
	2	163
	5	160
	10	163
	21	165
	25	163
554	0	106
	5	108
573	0	200
	5½	198
574	0	234
	2½	238
	6½	243
577	0	179
	6½	170
579	0	159
	5	161

*Aided by a grant from the Josiah Macy, Jr., Foundation.

given and at certain intervals after anesthesia was induced. Determinations of the total cholesterol were made on the serum according to the method of Bloor, Pelkan and Allen.[1] The dogs were in the fasting state in all instances except dog No. 304, which had received a high-cholesterol meal 2 hours before anesthesia was produced.

The results are shown in Table I.

Conclusions. Nembutal in amounts sufficient to produce deep narcosis lasting several hours in dogs is without significant effect upon the level of the total serum cholesterol.

8041 P

An Improved Method for Determination of the Gonadotropic Hormone.

U. J. SALMON* AND ROBERT T. FRANK.

From the Laboratories and Gynecological Service of the Mount Sinai Hospital, New York City.

The following method for the extraction of gonadotropic hormone from human blood is a modification and improvement of the acid alcohol method previously reported from this laboratory.[1]

With the old method only a faint Reaction I was demonstrable in 40 cc. of blood taken on the 8th to the 12th day of the cycle. With the new method it has been possible to obtain both strong Reaction I and III with the same amount of blood at a comparable point in the cycle.

Method. (1) 40 cc. of vein blood is mixed with 30 gm. of anhydrous sodium sulphate, stirred to dryness and pulverized. (2) 200 cc. of 60% alcohol, acidified with 50% acetic acid to pH 5, is then added to the powder and shaken for one hour in a mechanical shaker. (3) The mixture is centrifuged and the supernatant alcohol decanted and kept. (4) A similar second extraction of the residual sludge is made. (5) The 2 alcohol extracts are each concentrated by evaporation under a fan at room temperature to a volume of 50 cc. and then centrifuged. (The concentration can be

[1] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

*Joseph Brettauer Research Fellow in Gynecology.

[1] Frank, R. T., Goldberger, M. A., and Spielman, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 999.

hastened by vacuum distillation, temperature not to exceed 23°C.). (6) The precipitate and scum which separate during the concentration are removed and shaken for 10 minutes with 10-20 cc. of dilute NaOH, the final pH of the mixture not to exceed 8.5 (thymol blue) (A). (7) The two 50 cc. alcohol extracts are united and allowed to evaporate at room temperature (fan) almost to dryness (B). (8) The residue (B) is added to the aqueous alkaline extract of the first precipitate (A), then shaken and the pH of the whole adjusted to 8-8.5. The extract is then shaken vigorously for 5 minutes and allowed to stand for 10 minutes at room temperature (C). (9) The extract (C) now is centrifuged and the pH of the supernatant fluid is then adjusted with dilute acetic acid to 7-7.5 (using phenol red) (D). (10) The extract (D) (the total volume of which should not exceed 5 cc.) is then allowed to stand overnight in the refrigerator at a temperature of 35-40°C. Crystals of sodium sulphate separate out. The supernatant fluid is then poured off (E). (11) The final extract (E) is injected into an immature rat weighing 20 to 24 gm. in 5 divided doses, over 3 days. The animal is killed in 96-100 hours after the first injection. The intensity of the reaction is determined by examination of serial sections of the ovaries.†

8042 P

Gonadotropic Blood and Urine Cycles in Normal Menstruating Woman.

ROBERT T. FRANK AND U. J. SALMON.*

From the Laboratories and Gynecological Service of the Mount Sinai Hospital, New York City.

A method for the determination of gonadotropic factor in the blood of menstruating, non-pregnant women was published from this laboratory.[1] This method consisted in the extraction by means of 60% acid alcohol of the residual sludge of 40 cc. of blood desic-

† The method of assay and the clinical application of the test are discussed in the succeeding report.

* Joseph Brettauer Research Fellow in Gynecology.

1 Frank, R. T., Goldberger, M. A., and Spielman, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 999.

cated with anhydrous sodium sulphate. The estrogenic factor may first be removed with ethyl ether.[2]

By modifying the method it has been possible (a) to obtain a stronger follicle stimulating effect (Reaction I) and (b) to demonstrate a luteinizing effect (Reaction III) at certain periods in the cycle of non-pregnant, normal, menstruating women.

The cycle of excretion in the urine has also been studied, employing the Katzman-Doisy benzoic acid method.[3]

By means of these methods a normal woman has been studied throughout 3 menstrual cycles, and 3 other less regularly menstruating females have each been carried through one cycle of both blood and urine.

The maximum concentration of gonadotropic hormone in the blood occurred on the 9th to the 12th day. In this case Reaction I was obtained on the 9th day and Reaction III on the 12th day with 40 cc. of blood, giving a concentration of 25 R.U. of gonadotropic hormone per liter of blood. At other periods in the cycle, negative results were obtained with equal quantities of blood.

In the urine negative results with quantities up to 500 cc. were obtained except on the 10th to the 14th day of the cycle. During this period both follicle stimulating and luteinizing reactions were found with amounts varying from 40 to 500 cc. The concentration of the hormone in the urine was between 2 and 25 R.U.L. The maximum excretion in one day was 44 R.U. The total excretion in the first cycle amounted to 80 R.U. and in the second cycle to 54 R.U.

The method of assaying consists in injecting the extract in 5 divided doses over a 3-day period, into 20-24 gm. white female rats, the animals being killed 96 to 100 hours after the first injection. The ovaries are studied in serial section.

Ovaries containing from 2 to 3 large cystic follicles were recorded as faintly positive.

Ovaries honeycombed with large cystic follicles were recorded as a strong Reaction I.

Ovaries containing corpora lutea were tabulated as Reaction III.

Summary. By an improved method increased concentration of the follicle stimulating factor and the presence of luteinizing factor can be demonstrated in the blood of cyclical, menstruating women during the 9th to 12th day of the cycle. 2. In the urine a similar accumulation of both factors was noted between the 10th to the

[2] Frank, R. T., and Goldberger, M. A., *J. A. M. A.*, 1928, **90**, 376.

[3] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

14th day. 3. The presence of the luteinizing reaction has hitherto been associated with pregnancy and chorionepithelioma.

8043 P

Antibody Production by the Rabbit Against an Ectoparasite.

JAMES T. CULBERTSON. (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

The itch mite, *Psoroptes communis* var. *cuniculi,* is a common ectoparasite upon the rabbit, large numbers being found upon the skin surface deep within the fold of the outer ear. The skin where the mites occur becomes necrotic and is sloughed off. If a small piece of the slough be examined with a low-power microscope, many mites can be seen crawling over it.

It has occurred to the writer that repeated skin puncture by such numbers of ectoparasites, each injecting in the form of its saliva a small quantity of protein peculiar to the parasite, should lead to the production of specific antibody against the attacking species. We have endeavored to determine if this be true by testing for the presence of precipitin in 10 adult rabbits, 5 known to be infested with the parasites, these being observed in each case under the microscope, and 5 others apparently free of such infestation, neither the parasites nor their characteristic effects being detected upon careful search.

The antigen—an extract of the mite—was prepared in the following manner. Pieces of the sloughed skin were removed with forceps from the infested ears and placed in a Petri dish. As we observed them with a low-power dissecting binocular, the mites could be seen to leave the skin and crawl about over the glass plate. They were picked up, best in groups of several, on the point of a dissecting needle and transferred to a small mortar. After about 300 mites were thus transferred, they were mashed with a pestle and triturated for 10 minutes with 1½ cc. of Coca's solution. The mixture was then centrifugated at high speed and the *very slightly* opalescent supernatant fluid decanted. This fluid was used as the antigen.

In the precipitin tests, which were carried out in small-bore tubes, 0.1 cc. of the antigen was layered over an equal volume of the clear

serum of each rabbit studied. The tubes were incubated for one hour at 37°C. and read for "rings" at the interface of the fluids. The fluids were then mixed, incubated a second hour at 37°C., placed in the cold for 24 hours, and examined for precipitate.

It was found in these tests that only those animals which harbored the mites possessed demonstrable serum precipitin against an extract of the mite protoplasm. The serum of the uninfested rabbits gave no reaction with the mite extract. It is indicated, therefore, that infestation with this ectoparasite gives rise to a specific antibody in the blood of the infested rabbit.

In the light of this finding, it seems possible that the sloughing of the affected skin is essentially an Arthus reaction, resulting when the specific antigen of the mite saliva is introduced into the sensitized animal as the parasites bite in many places over a limited area. It is generally believed that the slough is a dried deposit of serum exuded from the area injured by the mites.

8044 P

Electrical Stimulation of a Nerve Muscle Preparation Without Contact.

RICHARD M. BRICKNER AND ROYAL E. GRANT. (Introduced by N. Kopeloff.)

From the Department of Neurology, Columbia University, New York, and the Neurological Institute, New York.

A method has been devised by which it is possible to stimulate a nerve-muscle preparation from a frog without contact between the tissue and the electrode.

The essential principle of the method is the placing of the nerve in the vicinity of, but insulated from, an induction coil. The coil is supplied with energy by an oscillator and suitable amplifier. The preparation is arranged in connection with a kymograph in the usual manner.

Two different exciting coils have been employed thus far. Each is wound with insulated copper wire upon a bakelite bobbin and in each the windings are infiltrated with bee's wax. Each is entirely enclosed in a bakelite case. The body of each contains a lumen, running horizontally through its center from end to end, large enough to admit the sciatic nerve of a frog. In the smaller of the

coils (coil 1) the lumen is lined with bee's wax. In the larger coil (coil 2), the lumen is large enough to hold a glass tube, into which the nerve is inserted. Coil 1 has about 500 turns of wire and coil 2 about 700. In coil 1 the minimum distance between the nerve and the nearest wire is about 1 mm.; in coil 2 it is about 2 mm. It will be seen that, in coil 1, nerve and wire are separated by bakelite and wax, and, in coil 2 by bakelite and glass. It has been found that good contractions could also be obtained with either coil, without inserting the nerve into the lumen at all, but by threading the nerve through a glass tube and strapping the tube across the bakelite front of the coil with tape. With this modification there is a distance of at least 3½ mm. between the nerve and the nearest wire: Precautions have been taken to protect the preparation from parasitic currents and from leakage. The generating unit can supply up to 150 milli-amperes to the exciting coil at the lowest frequencies. The frequency range is from 1 to 17,000 cycles per second. The field strength of the exciting coils has not, as yet, been measured.

With different specimens, and under various conditions, good contractions have been obtained at frequencies ranging from 100 to 1,000. Tetanus can be produced with single stimuli at frequencies usually between 500 and 1,000, or by gradually increasing the frequency while the current is running.

Although measurements of field strength have not been made thus far, it is plain from gross observations that the electromagnetic field is a fairly strong one. The strength of the electrostatic field is also still unknown to us. Up to the present it has not been possible to make observations sufficiently refined to enable us to tell how much of the effect is due to magnetic and how much to static stimulation, nor to enlighten us about the varying effect of changing conditions of all sorts. This communication is a statement of our first experimental results. A great deal of additional study is required before accurate interpretations can be made. It is hoped that further developments of them can be reported later. Experiments are being commenced to test out these and other relevant matters.

The investigation is also being continued with intact animals and with human beings.

Professor Horatio Williams has called our attention to some experiments performed 40 years ago by Professor Danilevsky. This investigator was able to produce contractions in frog nerve-muscle preparations without contact. He used a Ruhmkorff coil for stim-

ulation. His work appears never to have been developed, and to have been unfortunately lost.

This work has been conducted through the courtesy of the Department of Physiology.

8045 C

Detoxification of Amidopyrine by Sodium Amytal.

CHARLES L. ROSE. (Introduced by K. K. Chen.)

From the Lilly Research Laboratories, Indianapolis.

The antidotal action of sodium amytal against convulsant drugs has been demonstrated in both animals and men.[1–4] In view of the fact that large doses of amidopyrine cause convulsions of central origin,[5] it appears interesting to ascertain whether or not sodium amytal will similarly reduce its toxicity. Evidence of antagonism between amidopyrine and other barbituric acid derivatives has already been observed by several European workers.[6, 7]

White mice numbering 272 and rats numbering 221 were employed in the present investigation. They were all starved over night prior to medication. The drugs were injected by the tail vein. It is a coincidence that amidopyrine and sodium amytal when given alone have the same toxicity. The M.L.D. (minimal lethal dose) of each was found to be 150 mg. per kg. in mice and 135 in rats—using dose increments of 5 mg., and groups of 5 animals. The determined dose killed at least 3 animals out of an injected group of 5.

In the next series of experiments, sodium amytal was added and injected together with amidopyrine, various amounts of both drugs being used. As shown in Table I, the toxicity of amidopyrine is

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1 Knoefel, P. K., Herwick, R. P., and Loevenhart, A. S., *J. Pharm. and Exp. Therap.*, 1928, **33**, 265; 1930, **39**, 397.

2 Zerfas, L. G., and McCallum, J. T. C., *Anesth. and Analg.*, 1929, **8**, 349.

3 Swanson, E. E., *J. Lab. and Clin. Med.*, 1932, **17**, 325; 1933, **18**, 933.

4 Kempf, G. F., McCallum, J. T. C., and Zerfas, L. G., *J. A. M. A.*, 1933, **100**, 548.

5 Edmunds, C. W., and Gunn, J. A., *Cushny's Pharmacology and Therapeutics*, 10th edition, 1934, 553.

6 Käer, E., and Loewe, S., *Schmerz, Narkose, Anesth.*, 1929, **1**, 11; 1929, **2**, 323.

7 Pohle, K., and Spickermann, W., *Arch. f. exp. Path. u. Pharmak.*, 1931, **162**, 685.

TABLE I.
Detoxification of Amidopyrine by Sodium Amytal.

Species of Animals	No. of Animals Used	Amt. of Sodium Amytal Added mg. per kg.	M.L.D. of Amidopyrine mg. per kg.
Mice	52	0	150
	44	15	300
	35	30	420
	26	45	420
	54	60	480
	33	75	460
	12	90	460
	16	105	460
Rats	40	0	135
	46	13.5	320
	32	27.0	320
	17	40.5	320
	18	54.0	320
	42	67.5	400
	26	81.0	380

reduced approximately 2/3 when an optimal dose of sodium amytal was employed (which was 40% of the M.L.D. in mice, and 50% in rats).

It may be interesting to mention that in a group of 5 rabbits which was given by mouth 100 mg. of amytal and 227 mg. of amidopyrine daily, except Saturdays and Sundays, for 4½ weeks, there was no decrease in either the total white blood cell or the granulocyte counts. An examination of the microscopic sections of the bone marrow revealed no pathological changes. While these results definitely indicate that amytal and amidopyrine have little influence upon the leucocytes, they do not rule out the possibility that granulocytopenia may sometimes occur with the administration of amidopyrine in certain sensitive human individuals.[8]

[8] Reznikoff, P., *J. A. M. A.*, 1934, **102**, 2183.

8046 P

Action of Mercurial Antiseptics on Muscle Oxydase.

DAVID I. MACHT AND HILAH F. BRYAN.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

While studying the effects of various chemicals and toxins on the activity of freshly prepared muscle oxydase, the authors made some interesting findings regarding the behavior of certain inorganic and organic mercurial antiseptics. A modification of the Thunberg method of determining the decolorization of a standard solution of methylthionine chloride, or methylene blue, in specially constructed glass vacuum tubes was employed. After considerable experimentation the following procedure was found to give the most satisfactory and surprisingly uniform results. Leg and abdominal muscles were carefully dissected from adult white rats, which had been killed by arteriotomy (or cutting of the vessels of the throat), and minced with sharp scissors in a glass mortar. To several grams of this muscle was added an equal number of cubic centimeters of physiological saline. The whole mass was then ground up with clean sand in a porcelain mortar for half an hour and then strained through fine linen. In this way a uniform suspension of minute particles of muscle tissue in muscle juice and saline was obtained. One cubic centimeter of such freshly prepared muscle suspension was introduced with a pipette into a Thunberg tube. Two cubic centimeters of methylene blue solution were then added to the muscle suspension in the tube. This indicator solution was made of 8 parts of methylene blue, 1:2,000, and 6 parts of 0.1 molar solution of acid potassium phosphate. With a Cenco-Hyvac vacuum pump the air was exhausted from the tube, which was then placed in a water bath at 38°C.; and the time required to completely decolorize the solution was carefully measured. The vacuum in the Thunberg tubes was perfectly maintained by carefully greasing the stopcock with a specially prepared lubricant containing a little gutta-percha. Such a vacuum being maintained, very accurate readings could be repeatedly obtained with any one specimen of muscle juice. Chemical compounds to be studied were respectively added to tubes containing muscle suspensions and allowed to act for a definite period of time, after which the Thunberg solution was introduced and the test was performed in the manner described.

The effect of the inorganic mercury salts, mercuric chloride, mercuric iodide and mercuric oxycyanide on muscle oxydase was compared with that of 3 organic mercurial antiseptics prepared in these laboratories; namely, Mercurochrome or dibrom oxymercury fluorescein, Merodicein or monohydroxy mercury di-iodo resorcinsulphonphthalein, and Flumerin or the disodium salt of hydroxy mercury fluorescein. The effect on enzymatic activity of different concentrations of these chemicals, to which suspensions were exposed for varying periods, was carefully investigated. Table I illustrates the average of findings obtained with these mercurials and also the results of control experiments made with alcohol (70%), phenol and Liquor cresolis compositus, U.S.P. In connection with the present work, interesting studies were made with solutions of Mercurochrome to which minute quantities of inorganic mercury (mercuric chloride) had been added. By the Thunberg method even slight adulterations of such Mercurochrome solutions with inorganic salts could be readily detected, as may be seen from the table.

TABLE I.

Drug Used	No. of Exp.	Concentration 1 part in	Time of Exposure min.	Time Required to Decolorize Muscle Juice Without Drug min.	Time Required to Decolorize Muscle Juice With Drug min.
Mercurochrome	10	250	30	78	78
"	10	125	5	74	75
"	10	"	10	74	79
"	10	"	15	74	80
"	10	100	6	53	63
Merodicein	10	500	30	78	78
"	10	250	30	31	35
Flumerin	5	500	30	78	68
Mercuric chloride	10	5,000	30	78	91
" "	10	2,000	15	78	killed
Mercuric iodide	10	6,000	15	78	88
" "	10	2,000	15	78	killed
Mercuric oxycyanide	5	10,000	15	78	killed
Mercurochrome	5	250	60	63	64
Mercurochrome plus bichloride	5	10,000	60	63	180
Mercurochrome	5	250	10	31	34
Mercurochrome plus bichloride	5	20,000	10	31	43
Alcohol	5	70%	30	78	125
Phenol	5	250	30	78	82
Liquor cresolis comp.	5	"	15	78	killed
Oxyquinoline sulphate	5	1,000	15	31	51

Such a procedure offers a means of detecting solutions of spurious or fraudulent Mercurochrome which occasionally appear on the market. The organic compounds were found to be much less toxic for the enzymes than the inorganic mercurials. This inhibitory action or depressant effect of the various mercurials for the muscle enzymes does not run parallel to their antiseptic activity but is rather an index of their toxicity. Thus, Mercurochrome solutions, 1 :250 to 1 :100, while quite sufficient to destroy all bacteria, did not inhibit the action of muscle oxydase to any great extent. On the other hand, solutions of mercuric bichloride (1 :10,000 to 1 :5,000) were markedly depressant for the enzymes and after longer exposures killed them.

8047 P

Response of Adrenalectomized Rats to Phloridzination.

GERALD EVANS. (Introduced by C. N. H. Long.)

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

It was shown previously[1] that when fasted rats were kept for 24 hours at ½ atmosphere, glycogen equivalent to 34% of previously existing stores and not accounted for by decreases in other carbohydrate, was laid down. This phenomenon was found not to occur in adrenalectomized rats.

To test further the importance of the adrenals in the new formation of carbohydrate (a) intact rats, (b) adrenalectomized rats and (c) rats with bilaterally demedullated adrenals were given daily 50 mg. of phloridzin in olive oil subcutaneously, and the urinary glucose, non-protein nitrogen and ketones determined.

The success of the demedullation was checked subsequently by serial section; no accessory adrenals were found in these animals.

The results for all are given in Table I. To exclude moribund values those for animals which did not survive at least 36 hours beyond the period of observation were not used in averages given.

It will be seen that the excretion of sugar, nitrogen and ketones is much diminished in adrenalectomized rats. The excretory values (ketones excepted) for demedullated animals equalled or exceeded those of intact animals.

[1] *Am. J. Physiol.*, 1934, **110**, 273.

TABLE I.
Urinary Excretion in Phloridzinized Rats.
Mg./100 gm. rat/day.

	No. of Animals	Days Post-Oper.	1st day of fast — Dextrose	Nitrogen	Ketones	D/N	2nd and 3rd day of fast average — Dextrose	Nitrogen	Ketones	D/N
Intact	14	—	624	165	76	3.85	478	148	229	3.18
Adrenalectomized	8	4	345	114	23	3.05	178	68	41	2.65
Both Adrenal Medullæ Removed	3	16	730	189	35	3.86	485	164	126	2.98

The failure of the adrenalectomized animals to excrete larger amounts can not be laid to a general debilitating effect of the operation for at the commencement of the experimental period these animals had all gained slightly (aver. 4 gm.) over the pre-operative weight and their rectal temperatures were not below 37°C.; furthermore, as previously shown[1] such rats when fed have normal glycogen stores.

The D/N ratio for the second and third days of fasting is definitely lower in the adrenalectomized animals than in the normal group.

Conclusion. The breakdown of protein to form sugar which occurs on phloridzination of rats is greatly reduced in the absence of the adrenal cortex.

8048 P

Extent of Absorption of Alcohol at Various Intervals After Oral Administration.

R. N. HARGER AND H. R. HULPIEU. (Introduced by K. K. Chen.)

From the Department of Biochemistry and Pharmacology, Indiana University School of Medicine, Indianapolis.

As a result of calculations based on blood alcohol figures obtained after the oral administration of alcohol compared with similar figures following intravenous administration, Haggard and Greenberg[1] have concluded that a period of about 6 hours is required for

[1] Haggard, H. W., and Greenberg, L. A., *J. Pharm. Exp. Ther.*, 1934, **52**, 167.

complete absorption from the gastro-intestinal tract, and that therefore the blood alcohol drop during the first 6 hours following oral administration is not a reliable index of the rate of metabolism of alcohol by the body. On the other hand, Hanzlik and Collins[2] using intact segments of the intestinal tract, Voltz and Dietrick[3] from determinations of residual alcohol in the gastro-intestinal tract, and Mellanby,[4] Widmark,[5] and Jungmichel[6] using the time of appearance of the peak in the blood alcohol curves, all concluded that complete absorption requires much less than 6 hours.

Since the tissues and contents of the stomach and intestines will store some alcohol regardless of the method of administration, the quantity of unabsorbed alcohol in the gastro-intestinal tract at any interval should be represented by the excess above the concentration resulting from parenteral administration. Employing this principle, a series of experiments were run in which fasting dogs received 3 gm. of alcohol per kilo administered by stomach tube, the alcohol being diluted with 3 volumes of water. If vomiting occurred the results were discarded. At various intervals following administration, groups of the dogs were quickly killed by a blow on the head and the entire gastro-intestinal tract analyzed for its alcohol content by a method recently developed by one of us (R.N.H.).[7] Another group of dogs received the same dosage of alcohol by intravenous administration in the form of a 12% solution in saline. The intravenous administration occupied one hour and at the end of the second hour these dogs were killed and their gastro-intestinal tracts analyzed for alcohol. The average concentration of alcohol found in the gastro-intestinal tracts of the intravenous group was subtracted from the corresponding figures for the dogs receiving alcohol orally to give the concentrations of unabsorbed alcohol. Table I shows the results where the interval employed was 2 hours, and indicates that in most cases this interval is sufficient for practically complete absorption. Using this same procedure it was found that the percent of absorption for other time intervals was as follows: One-half hour, 49.6, 69.5, 46.5, 65.1; average, 57.7; one hour, 90.5, 70.1, 96.2, 97.2; average, 88.5; 1½ hours, 93.3, 91.7, 98.5,

[2] Hanzlik, P. J., and Collins, R. J., *J. Pharm. Exp. Ther.*, 1913, **5**, 185.

[3] Voltz, W., and Dietrich, W., *Biochem. Z.*, 1915, **68**, 118.

[4] Mellanby, British Medical Research Committee, Special Report Series, 1919, **31**, 1.

[5] Widmark, E. M. P., Die theoretischen Grundlagen und die practische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung, Berlin, 1932.

[6] Jungmichel, G., *Arch. f. exper. Path. u. Pharmak.*, 1933, **173**, 388.

[7] Harger, R. N., *J. Lab. and Clin. Med.*, 1935, **20**, 746.

TABLE I.

Comparison of Results after Oral and Intravenous Administration of Alcohol Showing Alcohol Remaining in Gastro-Intestinal Tract. Dose, 3 gm. per kg.; time, 2 hours.

	—Intravenous—		—— Oral ——			
	Dog 20	Dog 22	Dog 4	Dog 17	Dog 18	Dog 19
Wt. (kg.)	6.4	12.0	5.0	10.2	8.9	5.9
Alcohol administered (gm.)	19.2	36.0	15.0	30.6	26.7	17.7
Wt. of:						
Stomach contents (gm.)	4	8	146	31	156	13
" tissue "	78	125	69	120	144	96
†Gut, upper half "	141	184	324*	234	210	164
† " lower " "	114	219		135	175	146
Conc. of alcohol:						
Stomach contents (mg./gm.)	3.53	3.34	5.56	8.04	15.70	7.52
" tissue "	2.78	2.59	3.09	3.70	7.26	3.20
†Gut, upper half "	2.97	2.99	2.51*	3.13	3.28	3.18
† " lower " "	2.91	2.98		2.59	2.65	3.27
Blood	3.72	3.82	3.37	3.20	4.14	3.85
‡Unabsorbed alcohol:						
Stomach contents (gm.)			0.38	0.14	1.92	0.06
" tissue "			0.05	0.12	0.66	0.05
Gut, upper half "				0.04	0.06	0.03
" lower " "				—0.05	—0.05	0.05
Total unabsorbed "			0.43	0.25	2.59	0.19
% unabsorbed			2.8	0.8	9.7	1.0
% absorbed			97.2	99.2	90.3	99.0

†Including contents.

*Total gut and contents analyzed together.

‡ Weight of organ or contents X (concentration found minus average concentration from intravenous administration.)

90.0; average, 93.4. These figures do not support the conclusions of Haggard and Greenberg, whose calculations for absorption are: One-half hour, 48.8%; one hour, 67.5%; 1½ hours, 75.7%; and 2 hours, 83.7%.[8]

8049 C

Lipoid and Hemorrhagic Changes in Adrenal Cortex Following Traumatic Shock.*

J. KENNETH DONAHUE AND WILLIAM M. PARKINS. (Introduced by W. W. Swingle.)

From the Biological Laboratory, Princeton University.

It has been shown that if profound and fatal shock is to be obtained in the intact dog by traumatization, the severity of tissue

[8] Haggard, H. W., and Greenberg, L. A., *loc. cit.*, Fig. 2, Curve B.

* The expenses of this investigation were defrayed in part by a grant from the Rockefeller Foundation.

injury must be very much greater than is required to induce shock symptoms in the equally healthy and vigorous animal lacking adrenal glands.[1] Evidence is presented indicating that the extreme susceptibility of the adrenalectomized dog to shock following trauma is largely due to the absence of one of the mechanisms concerned with the maintenance of normal blood volume, namely, the adrenal cortex. Data are presented here which show that the adrenal cortex of intact dogs dying from traumatic shock presents histological evidence of considerable impairment.

The lipoid studies were made on frozen sections of the adrenals variously stained with 2% osmic acid, Scharlach R, and Sudan III. For the study of adrenal cortical hemorrhages and other histological details, paraffin sections were stained with iron hematoxylin and eosin.

The dogs in both the normal and experimental groups were rigorously selected and only mature, well-nourished animals were employed. Deep nembutal and ether anesthesia was used and care was taken that the animals suffered no pain. Shock was induced by traumatization of the muscles of the hind limbs.[1] Periodic blood-pressure readings were taken by direct needle-puncture into the femoral artery and used as a criterion for the presence and duration of shock.[2] Following the temporary fall and rise of blood-pressure characteristic of primary shock, a subsequent and more gradual fall in pressure to 50 mm., or less, indicated the unquestioned presence of secondary shock.

A definite depletion in adrenal cortical lipoid occurs in dogs dying from traumatic shock as shown in Table I. Three dogs (11T, 12T, 13T), whose blood-volumes were temporarily increased and survival periods lengthened by single intravenous injections of saline showed marked lipoid depletion of the adrenal cortex at autopsy. Two dogs (14T, 15T), receiving both saline and cortical hormone were relieved of shock symptoms but nevertheless showed marked depletion of cortical lipoid following a 2-day survival period. Apparently the rise in blood-pressure above shock level resulting from saline or cortical hormone administration, is not accompanied by an immediate restoration of lipoid in the adrenal cortex. Lipoid depletion was most marked in the reticular and inner fascicular zones of the cortex. Extreme depletion of lipoid from the glomerular zone was noted in the adrenals of the 2 dogs whose survival periods were lengthened by saline and cortical hormone.

[1] Swingle, W. W., and Parkins, W. M., *Am. J. Physiol.*, 1935, **111**, 426.
[2] Parkins, W. M., *Am. J. Physiol.*, 1934, **107**, 518.

TABLE I.
Lipoid Depletion in the Adrenal Cortex Following Trauma.

Dog No.	Survival after trauma	Glomerular lipoid	Outer fascicular lipoid	Inner fascicular lipoid	Reticular lipoid	Remarks
1N		+++	++++	++++	+++	Normal
2N		+++	++++	++++	+++	"
3N		+++	++++	++++	+++	"
4N		+++	++++	++++	+++	"
5N		+++	++++	++++	+++	"
6N		+++	++++	++++	+++	"
8T	5 hrs.	+++	+++	++	++	Trauma
9T	6 "	+++	+++	++	+++	"
10T	8 "	++	+++	++	+	"
11T	12 "	+++	++	++	+	" (saline)
12T	21 "	+++	++	++	+	" "
13T	9 "	+++	+++	+	+	" "
14T	47 "	+	+++	++	++	" " and extract
15T	46 "	+	+++	++	++	" " and extract

\+ practically no lipoid.
\+ + lipoid droplets all small.
+++ packed with small droplets plus some large droplets.
++++ packed with small droplets plus many large droplets.

Secondary shock may result in hemorrhagic changes in various organs,[3] including the adrenals.[4] Hemorrhages of the adrenal cortex have also been noted in the rat following pituitarectomy[5] and are said to occur following other conditions such as severe burns and the injections of various toxins. In view of the importance of the adrenal cortex as a factor in the maintenance of normal blood-volume[6] the occurrence of hemorrhagic changes in the adrenal cortex of the shocked dog becomes significant since such changes would undoubtedly interfere with its normal function. Table II indicates that hemorrhages may be expected to appear in the adrenal cortex of dogs 4 or more hours following trauma. Apparently the degree of trauma, rather than the survival period *per se,* determines the time of appearance and the severity of cortical hemorrhages. The hemorrhagic areas were usually confined to the reticular and fascicular zones. Occasionally hemorrhages were seen in the glomerular zone. The condition varied from a few blood points in the reticular zone to that pictured in Fig. 1, in which the entire reticular zone, a large part of the fascicular zone, and portions of the glom-

3 Moon, V. H., and Kennedy, P. J., *Arch. Path.,* 1932, **14**, 360.
4 Brooks, B., and Blalock, A., *Annals Surg.,* 1934, **100**, 728.
5 Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 655.
6 Swingle, W. W., Vars, H. M., and Parkins, W. M., *Am. J. Physiol.,* 1934, **109**, 488.

FIG. 1.
Hemorrhages of the adrenal cortex of a dog surviving 8 hrs. following very severe trauma. The dark areas represent gross hemorrhages.

erular zone, were hemorrhagic. In this, and other severe cases, hemorrhagic areas were also present in the medulla.

That the extent of hemoconcentration is reflected in the time of appearance and the severity of hemorrhagic changes in the adrenal cortex is illustrated in Table II. Dogs were subjected to intraperitoneal injections of glucose (100 cc. 5.5% solution/kilo) which was withdrawn by paracentesis after 5 hours.[7] The adrenals showed congestion of the capillaries of the cortex. In one case (28) receiving 1720 cc. of glucose slight, but definite, hemorrhages were seen in the cortex at the end of the 5-hour period.

Intraperitoneal injections of glucose followed by paracentesis after 3 hours and subsequent trauma caused gross hemorrhages of the adrenal cortex. The hemorrhages appeared sooner and were much more striking than those observed in dogs subjected to trauma alone. It is noteworthy that the dogs subjected to intraperitoneal glucose administration required considerably less traumatization for the induction of shock than those subjected to trauma alone. The marked hemorrhagic condition shown in Fig. 1 was occasionally obtained by the infliction of very severe trauma, whereas a comparable condition was commonly observed in glucose-injected dogs which were subsequently mildly traumatized.

[7] Gilman, A., *Am. J. Physiol.*, 1934, **108**, 662.

TABLE II.

Hemorrhagic Changes in Adrenal Cortex Following Trauma, Intraperitoneal Injections of Glucose, and Glucose Injections Plus Trauma.

Trauma only			Glucose*		Glucose plus trauma†		
Dog No.	Survival after trauma	Hemorrhages in cortex	Dog No.	Hemorrhages in cortex	Dog No.	Survival after trauma	Hemorrhages in cortex
20	5 hrs.	mild	26	congestion	31	3 hrs.	severe
21	6 "	none	27	"	32	6 "	"
22	8 "	severe	28	mild	33	5 "	"
23	5 "	mild	29	congestion	34	9½ "	"
24	11 "	"	30	"	35	4 "	"
25	4 "	slight			36	1 "	mild

* Glucose left 5 hrs. in peritoneal cavity.
† Glucose left 3 hrs. in peritoneal cavity.

In addition to the lipoid and hemorrhagic changes mentioned, evidence of nuclear vacuolization, particularly in the fascicular zone, and the presence of large numbers of mononuclear phagocytes were noted in the adrenals of the shocked dogs.

The data indicate that following trauma and the onset of secondary shock the adrenal cortex is subjected to severe functional strains leading to a marked depletion of lipoid, gross hemorrhages into the gland and vacuolization of cells. These changes are indicative of marked reduction in the functional efficiency of the cortex in this syndrome.

8050 C

The Endometrial Mole.

H. SELYE, C. HARLOW AND T. McKEOWN.* (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal.

It is well known since the classical experiments of Loeb that the uterine mucosa is sensitized by the corpus luteum hormone in such a manner that it reacts to traumatization with the formation of a deciduoma, a tumor closely resembling in its structure the maternal part of the placenta. It has been noted, however, that while deciduomata form readily during the first part of gestation, they will not form during the later stages of pregnancy although active corpora lutea are present in the ovary. We have repeated and confirmed these observations on rats, placing silk threads into the uterus as a

* National Research Council Scholar.

local irritant, and we found that deciduomata may only be produced if the threads are placed between the fifth and eighth day of gestation, while later on in pregnancy the trauma does not call forth any local reaction. This, however, does not mean that the uterus is insensitive to trauma during the second half of gestation, for in another group of animals in which we slit the uterus open on one side, instead of merely irritating it with thread, the mucosa showed a very marked and rather curious reaction here described briefly.

In 12 rats one horn of the uterus was slit open between the 12th and 13th days of gestation while the other horn with its gestation sacs was not touched. Biopsy specimens taken 2 days later showed that the uterine mucosa of these rats is much thicker on the traumatized side than usual, and histologically this thickening proves to be due to a hydrops of the stroma mucosae. Five days later the mucosa is enormously enlarged and transformed into a translucent gelatinous tumor with a more or less irregular surface. Histologically this tissue consists of hydropic connective tissue derived from the stroma mucosae, and—as Figs. 1 and 2 show—it resembles

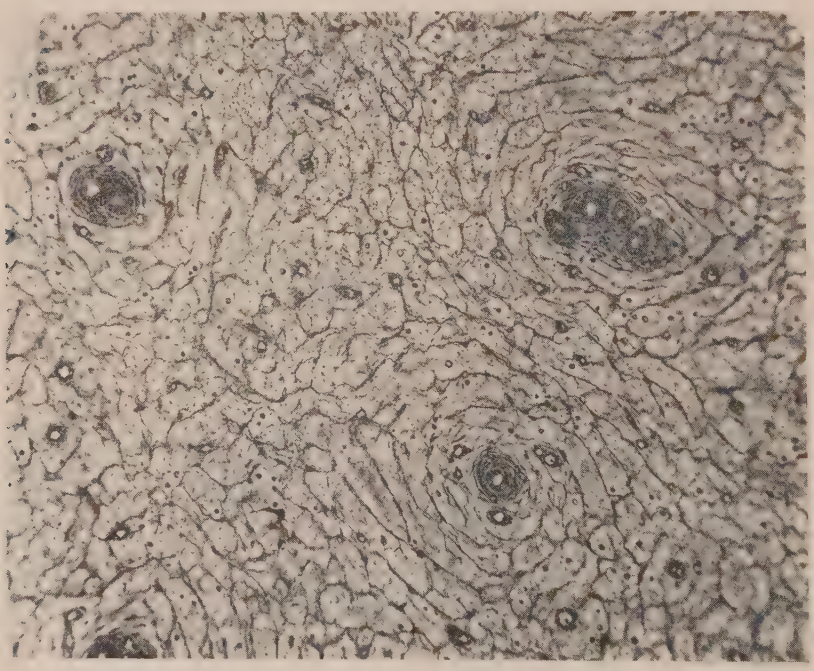

FIG. 1.
Low magnification of an endometrial mole showing the hydropic stroma and a few tubular glands in transverse section.

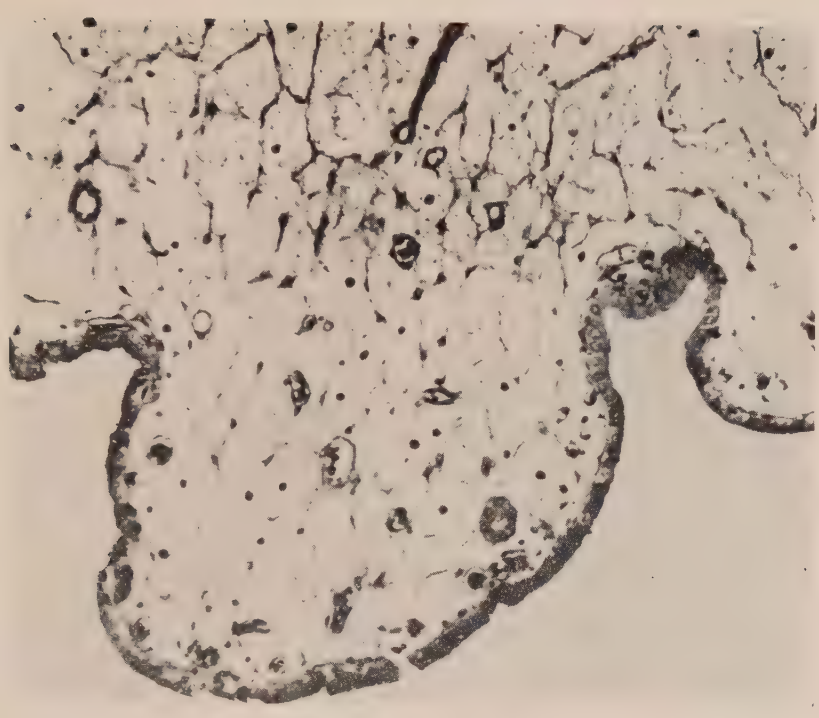

FIG. 2.
Endometrial mole at high magnification, showing the surface epithelium.

strikingly the connective tissue of a hydatidiform mole. The surface of the tumor is lined by the epithelium of the uterus. Since this tissue forms from the stroma of the endometrium and structurally resembles an hydatidiform mole we will refer to it in our discussion as the "endometrial mole" bearing in mind, of course, that there is no intimate relationship, other than the morphological similarity, between this structure and the hydatidiform mole, since the latter is derived from the foetal part of the placenta.

Since oestrin is known to inhibit deciduoma formation, we wished to see whether it would also interfere with the formation of these endometrial moles. In an experiment on 7 rats, the uterus was slit on one side between the 12th and 13th days of gestation and from then on the animals received 30 γ of oestrone, dissolved in corn oil, daily by subcutaneous injection. Biopsy on the 6th day after traumatization showed that far from being inhibited, the endometrial moles were larger in this group than in the previous one. These animals showed no vaginal oestrus. (The vaginal reaction was counteracted by the presence of the corpus luteum of gestation.)

It occurred to us that the ability of the uterus to form endometrial moles may be dependent on the simultaneous presence of oestrin and an active corpus luteum, and that the presence of oestrin, possibly formed by the placenta during the second half of gestation, may be responsible both for the formation of endometrial moles and for the impossibility to produce deciduomata at this period.

In order to subject this theory to experimental proof we treated a series of 4 lactating rats with 30 γ of oestrone daily beginning on the fifth day of lactation, at which date one horn of the uterus was slit, as in the previous experiments. Biopsy on the 5th day thereafter showed that large endometrial moles had formed under these circumstances but no deciduomata. Vaginal oestrus was not produced by this treatment owing to the presence of the corpus luteum of lactation. We might mention that control experiments showed that at this stage of lactation slitting of the uterus invariably leads to the formation of deciduomata in untreated animals. After this was established, we wanted to see how oestrin in itself would sensitize the mucosa of the uterus in the absence of corpus luteum hormone.

In 4 ovariectomized rats, treated with oestrone in the same manner as the animals of the previous series, the mucosa did not form large tumors after traumatism as it did in the experiments mentioned above, but microscopic examination showed marked hydrops of the mucosa. Since numerous control experiments have shown that no endometrial moles can be produced by trauma in the normally cyclic or ovariectomized female, it seems justified to conclude that the endometrial mole is a reaction to trauma dependent upon oestrin and particularly enhanced in the presence of an active corpus luteum. We think that this reaction is able to detect oestrin even though its vaginal effect be mashed by the simultaneous presence of an active corpus luteum. In other experiments we found that the daily administration of as large amounts as 2,000 to 3,000 γ of oestrone was unable to elicit the vaginal response in the presence of the corpora lutea of lactation or pregnancy, while as the present series shows, as small a dose as 30 γ produces unmistakable uterine reactions. The hypotheses that the corpus luteum hormone is responsible for the modification of the oestrin action, or that oestrone is transformed by the corpus luteum into a derivative with modified physiological effects, are equally compatible with our findings.

The formation of endometrial moles after trauma during the second half of gestation in the rat makes it very likely that oestrus-producing hormone is formed during pregnancy in the rodent.

8051 C

On a Peculiar Serum Protein, Precipitated by Hayem's Solution, Occurring in Multiple Myeloma.

BERNARD M. JACOBSON. (Introduced by L. Dienes.)

From the Medical Clinic, Massachusetts General Hospital, Boston.

During the estimation of the erythrocytes in the blood of a patient suffering from multiple myeloma, the author observed that immediately after the mixing of the blood with the Hayem's solution a coarse, white precipitate formed. This precipitate, when viewed under the microscope, consisted of amorphous, translucent masses. The same precipitate came down when either plasma or serum were mixed with Hayem's solution. Mixed with equal volumes of the diluent, dilutions of serum as high as 1:250 gave the same reaction. Sodium sulphate, which is present in Hayem's solution in a concentration of 2.5%, yielded, in aqueous solution, no precipitate with this serum, while bichloride of mercury, in a concentration of 0.25%, brought down the abundant precipitate.

Since the original observation, the writer has examined the bloods of 6 additional cases of multiple myeloma and has noted the above phenomenon in 3 instances, yielding a total of 4 cases, out of 7, which exhibited material in the blood that was precipitated by Hayem's solution.

In Tables I and II are presented data concerning some of the properties of the sera of 3 cases, and in addition of the serum of one case of myeloma in which the Hayem precipitate was absent.

TABLE I.

Results of the Fractionation of Myeloma Sera.

	Gm. of Protein per 100 cc. of Serum							
Patient No.	2	1				3	4	
Serum No.		3	4	5	Pericardial fluid		5	8
Total Protein	10.5	11.6	11.4	11.1	12.7	8.6	11.5	12.7
Albumin	1.6			2.2	3.6	2.0	0.0	0.0
Globulin	8.9			8.9	9.1	6.6	11.5	12.7
Euglobulin	3.1			7.9	7.9	4.8	9.7	10.0
Pseudoglobulin I and II	5.8			1.0	1.2	1.8	1.8	2.7
½ sat. $(NH_4)_2SO_4$ precipitate						7.0	11.5	
Sat. NaCl "						4.7		
CO_2 "	2.1			0.4	0.6	0.0		0.4
Sat. $MgSO_4$ "						6.2		

TABLE II.
Distribution Among Protein Fractions of the Hayem-Precipitable Material.

	Gm. of Protein per 100 cc. of Serum							
Patient No.	2	1*				3	4	
Serum No.		3	4	5	Pericardial fluid		5	8
Whole Serum	0	+	+	+	+	5.2	9.6	9.9
14% Na_2SO_4 Filtrate				±	±	0	0	0
18% " "				±		0	0	0
22% " "				0		0	0	0
½ sat. $(NH_4)_2SO_4$ Filtrate					0	0	0	0
Sat. NaCl Filtrate						0		
CO_2 "						4.4		9.9
Sat. $MgSO_4$ "						0		

*Only qualitative tests, for Hayem-precipitable material, were performed on the sera of this patient.

For the fractionation of the serum proteins the method of Howe[1] was followed; all determinations were performed in duplicate or triplicate.

From the data of Table I, it is apparent that in the sera of the 3 positive cases, the bulk of the globulin consisted of euglobulin, in contrast with the predominance of the pseudoglobulin fraction in the serum of the negative case, patient No. 2. Separation of globulin yielded, by means of ½ sat. $(NH_4)_2SO_4$, sat. NaCl, and sat. $MgSO_4$, values not far removed from those furnished by 22% Na_2SO_4, but practically none of the globulin in the 3 positive cases was brought down by CO_2. A study of the distribution of the material precipitated by Hayem's solution (Table II) demonstrates that it is brought down completely by globulin precipitants, with the exception of CO_2, and that it is almost completely confined to the 14% Na_2SO_4 precipitate, that is, to the euglobulin fraction.

It is evident, from the precipitation reactions, that the material which is brought down by Hayem's solution is not Bence-Jones protein, for the latter protein is not brought down by Hayem's solution or by sat. Na_2SO_4; nor is it precipitated by sat. NaCl or by sat. $MgSO_4$.[2]

Further study of the nature of this peculiar protein that is brought down by Hayem's solution must await the acquisition of a large amount of material.

[1] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 109.

[2] Magnus-Levy, A., *Z. physiol. Chem.*, 1900, **30**, 200.

8052 C

Rôle of Coagulating Principle of Staphylococcus Aureus in Relation to Invasiveness of this Microorganism.*

VALY MENKIN AND HENRY D. WALSTON.

From the Department of Pathology and the Department of Bacteriology, Harvard University Medical School.

Previous studies by one of us had shown that the localizing property of *Staphylococcus aureus* was referable to its ability to induce a powerfully injurious reaction in tissues inoculated with it. The severe injury to the capillaries, by increasing their permeability, doubtless allowed plasma proteins, including fibrinogen, to pass out into the inflamed area and be deposited there as coagulated plasma. The intense damage on the part of the pathogenic organisms to the draining lymphatics induced their early occlusion by the formation of fibrinous thrombi. The infected area became thus "walled-off" as early as one hour following the inoculation of the staphylococcus organism.[1] Trypan blue injected into such an area failed at this stage to diffuse to the tributary lymphatic vessels. Similar results were obtained with the Berkefeld filtrate of this microorganism.[2, 3] These results were in marked contrast to the delayed fixation of the dye found in the case of hemolytic streptococcus infection. In skin areas of rabbits inoculated with the latter organism, the lymphatic channels maintained their patency for almost 2 days before the inflammatory reaction became sufficiently intense to induce lymphatic blockage.[1] The conclusion was drawn that the *Staphylococcus aureus* is primarily a non-invading organism owing to its intense local effects which cause it to be circumscribed promptly by the formation of a mechanical barrier in the form of a fibrinous meshwork as well as thrombosed lymphatics in tissues distended with edema. The generalized systemic effects caused by the hemolytic streptococcus was referred to the relatively mild local effects produced by this organism which thus allowed its relatively free dissemination into the regional lymphatic vessels.

A number of authors including Much, Gratia, Gross, and Gengou, have shown that the *Staphylococcus aureus* organism and the filtrate

*This study was aided by grants from the DeLamar Mobile Research Fund and from the William W. Wellington Memorial Research Fund.

1 Menkin, V., *J. Exp. Med.*, 1933, **57**, 977.

2 Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 162.

3 Menkin, V., *Am. J. Med. Sci.*, 1935. In press.

of its culture are capable of causing oxalated blood to clot in the test tube.[4-7] Much called the clotting principle of *Staphylococcus aureus* a specific ferment which he designated *staphylokinase*. The material was found to be thermostable by Gratia, who gave it the name of *staphylocoagulase*.

Does "Staphylocoagulase" play a significant rôle in circumscribing a tissue area infected with *Staphylococcus aureus* by favoring the formation of a local fibrinous barrier? In other words, is the early fixation of trypan blue in the skin of rabbits treated with *Staphylococcus aureus* referable to the production of this clotting principle by the organism or is it due rather to the injurious action *per se* of the microorganism and its toxin? Evidence here presented definitely shows that fixation or the early "walling-off" reaction induced by staphylococcus has apparently no connection with the *in vitro* coagulating principle elicited by this microorganism or its products.

Gross had shown that the clotting reaction is readily elicited from the filtrate of a culture of *Staphylococcus aureus*.[6] For this reason all observations, with the exception of one experiment, were made on Berkefeld filtrates of a single strain of *Staphylococcus aureus* (strain 40). Most of the filtrates were obtained from 7-day cultures of the organisms in broth containing half the usual amount of meat and Parke-Davis peptone in place of the usual Difco peptone. Filtration was performed through a Berkefeld V filter. The filtrate inoculated in rabbit 11-86 was from a 20-day broth culture filtered through a Berkefeld N filter.

These filtrates were titrated in test tubes for their clotting potency. 1.5 cc. of the filtrate was injected intracutaneously in the foreleg of a rabbit. On the following day 2.5 cc. of 1% trypan blue in saline were injected into the inflamed area and a similar amount of dye was injected into the opposite forelimb to serve as control. Two to 3 hours later the regional lymphatics were exposed and studied for the presence of the dye by a technique previously described.[1, 8] It was found that by treating the filtrate of *Staphylococcus aureus* with glacial acetic acid the "staphylocoagulase" substance could be separated from the principle that induces fixation. The acetic acid treated fractions of the filtrates were obtained by

4 Much, H., *Biochem. Z.*, 1908, **14**, 143.
5 Gratia, A., *C. R. Soc. de Biol.*, 1920, **83**, 584.
6 Gross, H., *Z. f. Immunit.*, 1931, **73**, 14.
7 Gengou, O., *Ann. de l'Inst. Pasteur*, 1933, **51**, 14.
8 Menkin, V., *J. Exp. Med.*, 1929, **50**, 171.

adding to the filtrate 1/100 of its volume of glacial acetic acid and allowing this to stand overnight in the icebox. A precipitate formed. The supernatant fluid was decanted off and the precipitate taken up in its original volume of sterile distilled water; it was then brought up to a pH varying from 7.4 to 8.2 by the addition of N/20 NaOH. In this range the precipitate readily dissolved. The clotting capacity of a filtrate was found to be unaffected by varying its pH within the range described. Sterility was assured throughout by the addition of 0.5% phenol to the substances to be tested.

Titrations of the clotting properties of the substance to be tested were performed as follows: Blood was obtained from a normal rabbit by heart puncture, the sterile syringe already containing a small amount of sterile 4% sodium citrate in saline. The blood was centrifuged after sufficient citrate had been added to bring the total concentration to 2%; the supernatant citrated plasma was pipetted off with sterile precaution.

Dilutions of the "staphylocoagulase"-containing substance were made in small aseptic test tubes with sterile broth in the case of plain filtrates, and with sterile distilled water in the case of the acetic acid precipitates, the total volume being 0.5 cc.; to each dilution was added 0.5 cc. of the citrated plasma. The mixtures were then shaken and placed in a 37°C. incubator, and read at varying intervals, the final reading being taken after 48 hours, this having been found to give the most consistent measure of "staphylocoagulase". The end-point was taken as being that dilution where a definite clot, as opposed to fibrin threads, was formed.

The results of all the experiments are recorded in Table I. It is quite evident that the ability of trypan blue to be retained at the site of skin inoculated with either the filtrate of *Staphylococcus aureus* or its acetic acid precipitate bears no relationship to the *in vitro* clotting reaction. The filtrate of one strain of the microorganism produced fixation of the dye in the animal, although it failed to coagulate the plasma *in vitro* (Rabbit 11-86, Table I). The reverse state of affairs was brought about in the case of rabbit 11-46. The 2 phenomena of fixation and staphylocoagulase production are evidently entirely independent of each other. The intensity of one of these reactions does not in any way predict the trend of the other (*cf.* rabbits 11-86 and 11-46). Furthermore the acetic acid fractions indicate that although the fixation principle may be destroyed by chemical treatment, yet the *in vitro* clotting reaction persists strongly. The supernatant fraction of the acetic acid treated filtrate or the acetic acid precipitate of sterile broth produced neither

TABLE I.

In Vitro Clotting Principle of *Staphylococcus aureus* in Relation to Fixation of Trypan Blue at Site of Inflammation.

Rabbit No.	*Staphylococcus aureus* or its acetic acid treated fractions	Time between injection of filtrate (or fraction) and dye	Total duration of inflammation	Fixation of dye at site of inflammation	*In vitro* clotting reaction in 48 hr.
		hr.:min.	hr.:min.		
11-86*	1.5 cc. filtrate	21:05	24:00	+	0
11-46	1.5 " "	20:45	22:55	0	+++ (titer 1:620)
11-59	1.5 " "	21:10	22:50	+	+ (" 1:80)
11-52	1.5 " acetic acid precipitate	21:23	23:30	0	Fair (" 1:20)
11-53	1.5 " " " "	17:25	19:30	0	+
11-54	1.5 " " " "	18:50	21:00	0	+++ (" 1:320)
11-44	1.5 " " " "	21:00	23:20	0	++ (" 1:300)
11-57	1.5 " supernatant fraction of acetic acid treated filtrate	18:40	20:45	0	0
11-30	1.5 " acetic acid precipitate of sterile broth	21:00	23:20	0	0

*Filtrate of a 20-day culture of a strain of *Staphylococcus aureus* different from that used in remaining experiments.

fixation of the dye when inoculated into animals nor did it induce coagulation of citrated plasma in the test tube.

The "staphylocoagulase" precipitated from the filtrate of a culture of the microorganism when injected intracutaneously in a rabbit is absorbed from the skin by the tributary lymphatics, as indicated by the mild cellular reaction in the regional lymph nodes. Yet it fails to exert a sufficiently powerful local reaction to obstruct lymphatic drainage by the formation of a fibrinous barrier. Trypan blue diffuses readily from the site of its cutaneous inoculation and the tributary lymphatics are found unoccluded. These observations indicate that "staphylocoagulase" evidently plays no rôle in inducing prompt mechanical obstruction to lymph flow. The latter is therefore primarily referable to the powerfully necrotizing action *per se* of the *Staphylococcus aureus* microorganism and of its soluble toxin.

8053 P

Oxidation of Carbohydrates and Polyhydric Alcohols by Luminous Bacteria.

FRANK H. JOHNSON. (Introduced by E. Newton Harvey.)

From the Laboratories of General Physiology, Princeton University.

A comparative study of the oxidation of single substrates by the "resting" cells of the marine *(Achromobacter fischeri)* and the fresh water *(Vibrio phosphorescens)* luminous bacteria has revealed considerable difference in the ability of the 2 species to oxidize various carbohydrates and polyhydric alcohols. The rates of aerobic oxidation of 28 substrates by "resting" cells, prepared by washing thoroughly in the centrifuge and resuspending in phosphate buffer, were followed over a period of 3 hours in Warburg respirometers. The suspensions were adjusted to approximately the same density by means of a photronic cell turbiditimeter. The concentration of all substrates was M/60.

Although the per cent increase in oxygen uptake of the suspensions with substrate as compared with similar ones without the addition of substrate varied in some cases to a fairly wide degree, the ratio of the oxygen consumption with a given substrate to that with dextrose as a standard generally agreed to within less than 10% in repeated experiments. The controls, without added substrate, agreed with vessels containing substrates that were not oxidized to

TABLE I.
Aerobic Oxidation of Carbohydrates and Alcohols by "Resting" Cells of Luminous Bacteria.

Substrates Oxidized	*Vibrio phosphorescents*				*Achromobacter fischeri*			
	No. of determinations on different suspensions	Aver. mm³ O_2 per 3 hr.	Aver % increase over "resting" cells	Aver. ratio of O_2 consumption to that with dextrose	No. of determinations on different suspensions	Aver. mm³ O_2 per 3 hr.	Aver % increase over "resting" cells	Aver. ratio of O_2 consumption to that with dextrose
Dextrose	10	150.	516	1.0	9	101.	721	1.0
Fructose	5	108.	372	.79	2	95.	592	.90
Sucrose	3	101.	342	.70				
Trehalose	2	92.	330	.68				
Melicitose	2	87.	309	.65	2	65.	455	.61
Mannose	2	70.	209	.48	2	60.	367	.60
Glycerine	2	62.	192	.43	2	62.	381	.62
Maltose	2	49.	100	.34	1	39.	158	.37
Alpha galactose	3	43.	93	.32	2	50.	330	.51
Raffinose	2	34.	59	.28				
Sorbit	2	32.	41	.23				
"Resting" cells	10	22.		.16	9	12.		.12
Substrates not oxidized	d-Arabinose,* l-Arabinose, l-Rhamnose, d-Lyxose, Xylose, Ethylene Glycol, Dulcit, Quercit, Mannit, d-alpha-Galaheptose, d-beta-Galaheptose, d-alpha-Mannoheptose, d-Mannoketoheptose, Cellobiose, Turanose, Lactose, Kojic acid				d-Arabinose, l-Arabinose, l-Rhamnose, d-Lyxose, Xylose, Ethylene Glycol, Dulcit, Mannit, Quercit, d-alpha-Galaheptose, d-beta-Galaheptose, d-alpha-Mannoheptose, d-Mannoketoheptose, Cellobiose, Turanose, Lactose, Kojic acid, Sucrose, Trehalose, Raffinose, Sorbit			

*All the d- and l- forms of the sugars listed were kindly supplied by Dr. C. S. Hudson, of the National Institute of Health.

within 5% in the case of *Vibrio phosphorescens* though the agreement was not as close in the case of *Achromobacter fischeri,* in which the endogenous O_2 uptake was quite small and difficult to measure accurately.

The results are summarized in Table I. It is difficult to find any important correlations between the molecular configuration of the substrate and the ability of the organism to oxidize it. From the work that has been carried out thus far, however, it would seem that the primary limiting factor in both species is the number of carbon atoms, since only those compounds having 3 or 6 carbons are utilized. In the case of the disaccharides, those with beta linkages apparently are not utilized, while those with alpha linkages may or may not be utilized, irrespective of whether they are reducing sugars. (The type of linkage in sucrose, which is oxidized by the fresh water species, is not known.) Raffinose, which has a beta and a sucrose linkage, is oxidized only very slowly by the fresh water, and not at all by the marine species.

The results obtained by the respirometers were checked by acid production from the various substrates, under conditions of partial anaerobiosis. The method employed will be published shortly. The only discrepancy was in the case of glycerine, which is readily oxidized aerobically, but apparently not under conditions of limited oxygen supply.

8054 P

Effect of Experimental Hypothyroidism on Period of Gestation in the Rabbit.*

BORIS KRICHESKY. (Introduced by B. M. Allen.)

From the Department of Zoology, University of California at Los Angeles.

In some unpublished data obtained in this laboratory, it was found that thyroid removal in the pregnant female albino rat resulted in abortion or resorption of the foetuses, provided this operation was done before the first two-thirds of the gestation period.[1] Because the rabbit possesses a somewhat different type of ovarian activity than does the rat, it was thought to be of interest to determine

* Aided by a research grant from the University of California, and under the supervision of Dr. B. M. Allen. Appreciation is expressed to Mr. Allen Lein for technical assistance given.

1 Eckert, J. F., and Mazur, H.

whether the pregnant female rabbit would react to thyroidectomy in a similar manner. The results of this preliminary study indicate that contrary to the reaction obtained in the rat, thyroid removal has little if any effect on the period of gestation in the rabbit.

Although 33 New Zealand does, ranging in age from 7 to 18 months, were used as experimental animals, complete data on only 15 are reported here. The results on the remaining 18 animals will be reported when the data are completed. Ten additional normal females served as controls. Each doe was mated, being allowed to copulate twice, and the thyroid glands removed at definite intervals after mating. This interval in some cases was as short as 2 hours, while in other cases the time was extended to 12 days. The length of the period of gestation was then noted. The controls were mated but were not subjected to operative procedures and the duration of gestation also noted. As can be seen from Table I, no apparent differences between the length of the gestation period occurred in operated and unoperated animals.

It appeared possible that all of the thyroidectomies might not have been complete, and that regeneration of thyroid remnants might have occurred. In 6 animals (Nos. 3, 7, 8, 34, 40, and 54) which were sacrificed, the entire thyroid region was removed, fixed, and a

TABLE I.

Effect of Thyroid Removal on Period of Gestation and Rate of Oxygen Consumption in Rabbits.†

Animal No.	Interval between mating and thyroidectomy	Days gestation	Remarks	Rate of O_2 consumption per kilo. per hr. compared with normal (%)
45	2 hr.	31	4 young	69
54	6 "	30	3 "	78
40	6 "	31	4 "	70
32	10 "	—	Not preg.	79
39	10 "	32	5 young	71
49	1 day	31	4 "	67
34	1 "	30	?	86
41	1 "	—	Not preg.	70
7	2 "	30	4 young	73
8	2 "	31	5 "	78
36	2 "	—	Not preg.	—
11	4 "	—	Died 31 days after mating. Uteri contained 8 young	—
6	11 "	30	11 young	80
3	12 "	33	3 "	72
5	12 "	32	6 "	81

†Ten normal female rabbits used as controls showed an average period of gestation of 31 days with a variation from 29 to 32 days. The normal rate of oxygen consumption, taken as 100%, was determined from the average rate of 10 normal, non-pregnant females.

careful search made for traces of thyroid tissue under a binocular dissecting microscope. In animal No. 54, one relatively small piece, and in animal No. 34, two small pieces of thyroid material were found. In the remaining 4 animals no traces of the gland tissue were observed.

That these animals were definitely hypothyroid is shown by the decrease in their rate of oxygen consumption per kilogram per hour. This rate was determined by a modified Benedict apparatus for 10 normal, non-pregnant rabbits, and the average for the group was taken as normal. The decrease, determined one month after delivery of young and shown in the table, varied from 14 to 33%, indicating that all experimental animals exhibited some degree of hypothyroidism.

These results are not in agreement with the work of Ukita, who reported an increase in the period of gestation to from 60 to 70 days in rabbits thyroidectomized from the 7th to 10th day of pregnancy.[2] Our results indicate that experimental hypothyroidism produced after mating apparently had little effect on the duration of the gestation period in the rabbit.

8055 P

Growth Factors in Relation to Development of Certain "Fastidious" Bacteria.

STEWART A. KOSER AND FELIX SAUNDERS.

From the Departments of Hygiene and Bacteriology and of Physiological Chemistry, University of Chicago.

It is well known that many of the more fastidious bacteria require special media such as fresh meat infusions for their development and refuse to grow, or at best grow poorly, on the ordinary standard agar and broth made with peptone and meat extract. These organisms, almost without exception, also refuse to grow in a chemically definite or synthetic medium. The nature of the necessary substances in the more complex media is largely a matter of conjecture at present, although it constitutes one of the very important problems of bacterial nutrition.

In the present investigation we have attempted to separate from

2 Ukita, T., *Physiol. Abst.*, 1920, **5**, 39.

ordinary veal infusion the factors responsible for growth of some of the more exacting bacteria. It seems reasonable to suppose that a more exact knowledge of these substances would not only reveal many practical applications in bacteriological work but would also have a rather general biological significance.

The removal of growth factors from protein digests or infusions by the use of charcoal has been reported by Robinson and Rettger,[1] Thjotta and Avery,[2] Mueller,[3] and Freedman and Funk.[4] In our work it was found that treatment of an ordinary veal infusion with charcoal resulted in the removal of unknown substances necessary for the growth of certain microorganisms. Other commonly used adsorbents, such as Fuller's earth, kaolin, talc, Lloyd's reagent, filter paper, calcium phosphate, Supercel, or silica gel were relatively ineffective. The unknown substances or growth factors, after removal by charcoal from an infusion, could be recovered by extraction from the charcoal with hot ethyl alcohol or hot acetone.

These extracts, after evaporation of the alcohol or acetone and subsequent solution of the residue in water, were effective in "activating" an infusion from which growth factors had been removed,

TABLE I.
Addition of Growth Factors to Synthetic Medium and to a Mineral Salt Solution.

Organisms used	Extract added† cc.	Development in synthetic medium — days			Development in mineral solution (control) — days		
		1	2	7	1	2	7
Corynebacterium diphtheriae, Park 8	.1	—	+	+++	—	—	+
	.01	—	+	+++	—	—	—
	.001	—	—	++	—	—	—
	.0001	—	—	—‡			
	.00001	—	—	—			
	none	—	—	—	—	—	—
Corynebacterium diphtheriae, No. 5	.1	—	++	+++	—	—	—
	.01	—	++	++	—	—	—
	.001	—	—	+			
	.0001	—	—	—			
	none	—	—	—	—	—	—

Inoculations were made from salt solution suspensions of each organism, all tubes receiving 0.1 cc. The suspensions were prepared by lightly inoculating the salt solution from 24- or 48-hour growths on veal infusion agar.

†Amount of extract added to each 5 cc. tube of synthetic medium or mineral solution. The total solids, both organic and mineral, in this sample of extract amounted to 3 mg. per cc. of extract.

‡ In this case ++ growth had appeared after 14 days.

[1] Robinson, H. C., and Rettger, L. F., *J. Bact.*, 1918, **3**, 209.

[2] Thjotta, T., and Avery, O. T., *J. Exp. Med.*, 1921, **34**, 97.

[3] Mueller, J. H., *J. Bact.*, 1922, **7**, 309, 325.

[4] Freedman, L., and Funk, C., *J. Metabolic Res.*, 1922, **1**, 457.

or in rendering a standard beef extract-peptone broth more suitable for growth of certain exacting organisms.

The presence of essential growth factors in these extracts could also be shown by their addition to a synthetic medium containing several amino acids, dextrose, and mineral salts. An experiment of this nature is shown in Table I.

In the synthetic medium alone the diphtheria bacilli refused to develop. However, with the addition of small amounts of material extracted from veal infusion good growth was obtained. The amino acids and glucose were used as food and energy sources only when an additional factor was also present.

The total solids in the added extract amounted to only 3 mg. per cc. of extract. Thus, when 0.001 cc. or 0.0001 cc. of extract was added to a tube (5 cc.) of synthetic medium, the added total solids amounted to 0.0006 or 0.00006 mg. per cc. of synthetic medium. These small amounts of added material determined whether or not the diphtheria bacilli were able to develop in a synthetic medium which itself contained 5.3 mg. of organic matter per cc., or roughly ten thousand to one hundred thousand times as much. The extract evidently supplied very little actual food material, for when added to the mineral solution which was used for control purposes, a scanty, delayed growth occurred only where larger amounts, 0.1 cc. per tube, were used.

Quite similar results were obtained with several strains of dysentery bacilli, the typhoid bacillus and *Staphylococcus albus*. On the other hand, several strains of scarlet fever and septic sore throat streptococci, *Streptococcus lactis,* pneumococci and Pasteurella all refused to develop in the synthetic medium plus added extract. Further work showed that the factors responsible for growth were destroyed on ignition. A measured sample of extract was evaporated to dryness and the organic matter burned off. The ash which remained exerted no stimulating effect whatever.

The growth factors dealt with here are believed to belong in the class of growth accessory substances. Their addition to synthetic media, even though in the present impure state, presents a method for securing growth of at least some of the more fastidious bacteria in very simple culture solutions. The use of such solutions may offer another approach to the study of toxins and many other problems connected with the metabolism of these organisms.

8056 P

Sources of Growth Factors Required by Certain "Fastidious" Bacteria. Failure of Ascorbic Acid to Replace Growth-Promoting Principles.*

STEWART A. KOSER, FELIX SAUNDERS, ISADORE I. FINKLE, AND R. C. SPOELSTRA.

From the Departments of Hygiene and Bacteriology and of Physiological Chemistry, University of Chicago.

In continuing the study of substances, apparently in the nature of accessory growth factors, which will permit the development of some of the more exacting pathogens in a synthetic medium, it seemed desirable to gain some information concerning the natural sources of such compounds before attempting further chemical purification. A number of different animal and plant tissues were selected as possible sources and tested for the presence of growth promoting principles. The tissues were finely ground, extracted with water and heated to boiling. The coagulum was removed by filtration and the filtrate treated with charcoal to adsorb the growth factors. The charcoal was then extracted in a continuous extractor with hot ethyl alcohol to remove the growth factors, the alcohol evaporated off *in vacuo* and the residue taken up in a measured amount of distilled water. The extracts were sterilized in the autoclave.

The potency of these extracts was tested by adding them in several different dilutions to tubes of synthetic medium and noting the growth which followed inoculation with organisms which ordinarily refused to develop in the synthetic medium. Ten different cultures were used: *Streptococcus hemolyticus* (from scarlet fever), *Streptococcus lactis, Staphylococcus aureus, Staphylococcus albus, Corynebacterium diphtheriae, Brucella abortus, Pasteurella avicida, Salmonella pullorum, Eberthella typhosa,* and *Shigella dysenteriae.*

Observations of growth were made at several different intervals over a period of 10 days or occasionally longer. The rapidity of development of the organisms as well as the dilution in which growth occurred served to give a measure of the potency of the extracts. Altogether over 30 preparations were tested in this manner.

The results may be summarized as follows:† Veal infusion ++++,

* This investigation was aided by a grant from the Logan Fund.

†Comparative potency of the preparations is shown by the plus signs: ++++ very good, +++ good, ++ fair, + poor, and — no effect.

skim milk +++, calf liver ++++, ash from calf liver —, calf thymus +++, calf heart ++, calf spleen ++++, calf kidney +++, calf lung +++, pig embryo ++, chicken liver +++, shad roe +++, human liver with carcinoma +++, human placenta +++, human urine +, oat sprouts ++, wheat germ ++, rice bran +++, tea —, lettuce ++, sprouted soy beans +++, canned tomato juice +++, turnips +++, whole white potatoes +++, ash of potatoes —, potato sprouts +, potato tuber left after removal of sprouts ++, carrots ++, cocoanut milk +++, baker's yeast ++++, several cultures of Aspergillus and Penicillium + or ++, *Pseudomonas fluorescens* +.

In considering this summary it should be pointed out that the results do not necessarily give a quantitative picture of the relative amounts of growth factor actually in the products. Various considerations such as ease of extraction by the method employed, the possible presence of growth inhibiting substances or substances which might inactivate the growth factor, and the presence of varying amounts of extraneous material might affect the final results. Despite these uncertainties the results appear to have some value in showing the general distribution of growth-promoting principles and also in affording some idea of those products which might be used as sources of material for further attempts at chemical separation and purification.

It appeared of interest, also, to attempt the substitution of purified products for the foregoing extracts of animal and plant tissues. A sample of Merck's ascorbic acid (cevitamic acid) was available. In view of the established relationship of this to vitamin C of animal nutrition it was tested for any effect it might have on the growth of bacteria. The ascorbic acid was added to the synthetic medium used in the previous tests in amounts to give 0.1, 0.01, and 0.001 mg. per cc. of medium. The tubes were then inoculated with the same 10 microorganisms used for the previous tests. However, growth was never observed in any case and ascorbic acid could not be substituted as a growth-stimulating agent for the unknown products extracted from animal and plant tissues.

8057 C

Absorption of Carbon Particles from Gastro-Intestinal Tract.

F. S. CUTHBERT AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

From the literature,[1-5] it is clear that if carbon particles (India ink) in relatively large quantities are placed directly into a "fasting" loop of the intestine of a guinea pig, rabbit or dog, some absorption of the particles results. The literature is not clear in regard to whether carbon particles are absorbed when mixed with food. This work was undertaken to answer that question for dogs.

Adult dogs of average size were chosen. They were fed the regular stock diet of the laboratory which consisted of ground meat and bread. Fifty grams of "fine" lamp black were mixed daily with the ration. The animals relished the mixture regardless of its color. Five dogs were used; they were chloroformed at various stages—dog 1 at 16 days, dog 2 at 30 days, dog 3 at 2 months, dog 4 at 4 months, and dog 5 at 7 months after the feeding was begun. The mesenteric lymph glands, spleen and liver were sectioned and examined for carbon particles.

Black pigment was observed readily in the mesenteric lymph glands in 3 of the 5 dogs, dogs number 2, 3, and 4, it being more marked in dogs 2 and 4. On histologic examination the glands of all 5 dogs were found to be pigmented, those of dog 1 being the least pigmented. Dog 5 which received the carbon for 7 months contained only a little more pigment than dog 1; however, in this dog some black pigment was found in the spleen and the Kupfer cells of the liver. The pigment particles in the mesenteric lymph glands were located in both the cortex and medulla of the gland.

From these experiments it is apparent that, under the conditions stated, carbon particles are absorbed from the gastro-intestinal tract of the adult dog. It must be remembered, however, that a very large amount of lamp black was fed daily, so that there was a more

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1 Aufrecht, *Die Lungenentzundungen in Nothaagels spec. Path. und Ter. Bd.*, XIV.

2 Kuss and Lobstein, *Bull. Med. Par.*, 1907, **21**, 83; *Compt. Rend. de la Soc. Biol.*, 1907.

3 Ravenna, *Gazz. d. osp. Milano*, 1907, **28**, 177.

4 Chinaglia, *Atti d. r. Ist. Veneto di sc., lett. ed arti* (pt. 2, disp. 4), 1928-29, **88**, 337.

5 Tychowski, *Compt. Rend. de la Soc. Biol.*, 1930, **104**, 538.

or less constant inundation of the intestine with carbon particles. Further, the animals were kept on the described regime from 16 days to 7 months, a much longer time than that reported by other workers.

8058 P

Formation of Carbohydrates from Non-Glycerol Fraction of Lipoids in the Rat.

I. ARTHUR MIRSKY AND SAMUEL SOSKIN.

From the Metabolic Laboratory, Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

Although the study of the metabolic disturbance in the depancreatized dog has contributed in a large measure to our present knowledge of metabolism, there is still no universal agreement as to the exact nature of this disturbance. Two main schools of thought exist on this question. The theory which gained early acceptance in this country postulates an inability of the diabetic tissue to utilize carbohydrate. The opposing theory, more widely accepted in Europe, and gaining more credence in this country, states that the diabetic organism can utilize carbohydrate but that the essential disturbance is an overproduction of sugar from non-carbohydrate sources. A necessary corollary to this latter theory is a formation of carbohydrate from the non-glycerol fraction of lipoids.

We have previously reported that carbohydrate is utilized by the completely depancreatized dog not receiving insulin,[1] and many older and more recent reports may be drawn in support of this thesis.[2, 3, 4] The existing evidence for the conversion of fat to carbohydrate in the higher animal species is largely based on the D:N ratio and Respiratory Quotient, and is disputed on theoretical grounds,[5] although it is conceded that this conversion occurs in plants and probably also in insects. The present preliminary report offers direct proof, of a crucial nature, that carbohydrate can be derived from the non-glycerol fraction of lipoids in the rat.

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1 Soskin, S., *J. Nutrition*, 1930, **3**, 99.

2 Houssay, B. A., *Endocrinology*, 1931, **15**, 511.

3 Mann, F. C., *Archives Int. Med.*, 1923, **31**, 797.

4 Hedon, L., *Arch. Internat. de Physiol.*, 1926, **26**, 329; 1926, **27**, 254; 1927, **29**, 175.

5 Soskin, S., *Biochem. J.*, 1929, **23**, 1385.

Magne, Mayer and Plantefol[6] observed a marked depletion of the tissue carbohydrates in animals following the administration of dinitrophenol. Later, Dodds and Greville,[7] working with isolated tissues, concluded that the increased metabolism following dinitrophenol administration occurred solely at the expense of carbohydrates. It was difficult to reconcile these results with the work of Hall, Field, *et al.*,[8] who observed a significant decrease in the Respiratory Quotient of animals treated with dinitrophenol, from which they concluded that the fats were the chief source of the liberated energy. In some preliminary experiments in which we attempted to find an explanation for the above discrepancy, we found that while the oxygen intake in the intact dog given dinitrophenol was far in excess of that required to oxidize the carbohydrate which disappeared, the oxygen intake of hepatectomized dogs under the same conditions was less than the amount necessary to oxidize the disappearing carbohydrate. These results enabled us to reconcile the previous work by assuming that while the increased heat production caused by dinitrophenol occurred at the expense of carbohydrate, there also occurred a concomitant production of the necessary carbohydrate from other sources. Since it had been established that even prolonged administration of dinitrophenol does not increase the urinary excretion of nitrogen nor the non-protein nitrogen of the blood,[8] the gluconeogenesis presumably occurred at the expense of fat. The following experiments were therefore performed with a view to the possibility that following the height of the action of dinitrophenol, the rate of formation of new carbohydrate might be sufficiently greater than the rate of its oxidation to permit of the unequivocal demonstration of the former process.

In a number of sets of 2 litter-mate rats of approximately the same weight and fasted 24 hours prior to the experiments, 20 mg. per kilo of 2:4 sodium dinitrophenol was administered subcutaneously in 0.1% solution. In from one to 3 hours after dinitrophenol administration, one rat from each set was killed and passed through a meat-grinder. Aliquots of the thoroughly mixed, minced tissue were taken for the determinations of glycogen, soluble carbohydrates and lactic acid, by the procedure described by Dann and

[6] Magne, H., Mayer, A., and Plantefol, L., *Ann. de Physiol. Physicochim. Biol.*, 1932, **8**, 50.

[7] Dodds, E. C., and Greville, G. D., *Nature*, 1933, **132**, 966.

[8] Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432.

Chambers.[9] Total lipoid content was determined by a preliminary extraction with an alcohol-ether mixture and the weighing of the residue of a subsequent petroleum-ether extract.[10] Total nitrogen was determined by the Kjeldahl method and total solids by drying the tissues to constant weight at 80°C. Three to 4 hours later, the second rat of each set was killed and treated in a similar manner. All determinations were made on triplicate tissue aliquots, analyzed in duplicate, and calculated on the basis of dry weight.

TABLE I.

Rat No.	First Rat					Second Rat				
	Carbohydrate*					Carbohydrate*				
	Glycogen Mg. %	Soluble CHO Mg. %	Lactic Acid Mg. %	Total Mg. %	Total Lipoids Mg. %	Glycogen Mg. %	Soluble CHO Mg. %	Lactic Acid Mg. %	Total Mg. %	Total Lipoids Mg. %
1-2	378	145	1859	2382	18130	384	181	2602	3167	15694
7-8	300	197	488	985	16630	429	255	624	1308	13610
9-10	413	131	362	906	16000	469	241	465	1175	14250
13-14	399	120	360	879	14230	657	166	405	1228	12200
Total	1490	593	3069	5152	64990	1939	843	4096	6878	55754
% change									+33%	—14%
3-4	585	141	620	1346	17900	334	154	110	598	17300
11-12	250	262	524	1036	15500	255	153	454	862	15600

*Calculated as Glucose All calculations on basis of dry weight of tissue.

Some of our results are given in Table I. It may be seen that in those experiments in which we succeeded in observing the appropriate phase (above heavy line), there was a consistent and significant rise in the total carbohydrates (av. 33%) and a corresponding diminution in the total lipoids (av. 14%) of the second rat as compared to the first. When no increase in carbohydrate occurred (below heavy line), no fall in the total lipoids was found. No significant difference between total nitrogen content of the rats was observed. It is obvious that the glycerol fraction of the fat which disappeared cannot account for the total amount of carbohydrate which appeared since that would necessitate the disappearance of more than four times the amount of lipoid which is actually lost. The carbohydrate must, therefore, be derived from a fraction of the lipoids other than, or in addition to, glycerol.

9 Dann, M., and Chambers, W. H., *J. Biol. Chem.*, 1932, **95**, 413.

10 Chen, J. S., *Chinese J. Physiol.*, 1934, **8**, 195.

8059 P

Effect of Physical Training on Blood Volume, Hemoglobin, Alkali Reserve and Osmotic Resistance of Erythrocytes.*

JOHN E. DAVIS AND NATHAN BREWER. (Introduced by A. J. Carlson.)

From the Department of Physiology, University of Chicago.

That previously confined dogs when exercised for a few days undergo a loss of blood volume erythrocytes and hemoglobin has been shown by Broun.[1, 2] This loss he attributes to a process of fragmentation which other workers[3] have advocated as a normal mode of erythrocyte destruction. Thörner[4, 5] has reported an increase in the osmotic resistance of erythrocytes of man and dog after a period of training. This he explains by a "weeding out" through mechanical fragmentation of less resistant corpuscles. Hastings[6] has shown that *in vivo* hemolysis occurs in the serum of untrained dogs after exercise. Broun's dogs showed recovery of hemoglobin and erythrocytes with continued training and of blood volume after 3 weeks' rest. Anoxemia by anemia has been suggested as the bone marrow stimulant in the recovery process, by Steinhaus.[7]

The present work was undertaken to determine whether or not prolonged physical training will induce an increase of blood volume and erythrocytes, and if possible, to throw light on the hematopoietic stimulant as well as the mode of blood destruction in exercise.

Five dogs were confined for 2 or 3 months without exercise and were then exercised for 2 hours daily over periods of 4 to 9 weeks' duration. Observations on the blood were made throughout this time and continued for a few weeks after exercise was stopped. Two dogs swam in water at 30°C. for 2 hours and 3 ran on a treadmill set at 25% grade for a distance of 6 miles, daily. A constant mixed diet including 200 gm. of beef liver was fed daily beginning a month before control observations began and continuing throughout the exercise and post-exercise period. During the training period dogs were fed after the day's exercise.

* The present investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

1 Broun, G. O., *J. Exp. Med.*, 1923, **37**, 207.

2 Broun, G. O., *J. Exp. Med.*, 1923, **37**, 187.

3 Rous, P., and Robertson, O. H., *J. Exp. Med.*, 1917, **25**, 651.

4 Thörner, W., *Arbeitsphysiol.*, 1929, **2**, 116.

5 Thörner, W., *Arbeitsphysiol.*, 1932, **5**, 516.

6 Hastings, A. B., *U. S. P. H. Bulletin*, 1921, **117**, 17.

7 Steinhaus, A. H., *Physiol. Rev.*, 1933, **13**, 119.

All blood determinations were made under basal conditions of the animal at least 18 hours after previous exercise and food. Blood volume was measured by carbon monoxide inhalation method[8] using the blood gas manometric method of Van Slyke and Neill.[9] Hemoglobin was determined by the method of Palmer,[10] and alkali reserve was calculated as plasma bicarbonate using the CO_2 method of Van Slyke.[9] We used Hastings'[6] method for the measurement of erythrocyte osmotic resistance.

An increase in alkali reserve was finally obtained in all 4 dogs used for this test. Increased alkali reserve in training has been observed in man,[11] but not to our knowledge, heretofore, in dogs.

The osmotic resistance of erythrocytes increased significantly in the 2 swimming dogs, although 1 dog did not show any change after one week of training. Dog No. 5, a treadmill runner, used only for this test, showed a small decrease in R.B.C. resistance accompanied in the first week of training by a reduction of 15% in hemoglobin per unit volume of blood.

Blood volume, total cell volume, and erythrocyte count, with one exception, were sharply reduced in the first week, but in spite of continued exercise recovered to and exceeded the normal levels. These elevated values were observed in both running and swimming dogs and persisted usually for about a month after cessation of exercise.

Similarly, hemoglobin per unit volume of blood was at first sharply reduced by exercise, but recovered irregularly and did not in any case significantly exceed the normal level.

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8 Chang, H. C., and Harrop, G. A., *J. Clin. Invest.*, 1928, **5**, 393.

9 Van Slyke, D. D., and Neill, J. J., *J. Biol. Chem.*, 1924, **61**, 523.

10 Palmer, W. W., *J. Biol. Chem.*, 1918, **33**, 119.

11 Walinski, *Veröffentl. a. d. geb. d. Heeressanitätswesens*, 1925, **78**, 37.

8060 C

Responses of Mammalian Nerve to Strong Shocks.

GEORGE H. BISHOP* AND PETER HEINBECKER.

From the Laboratory of Neurophysiology and Department of Surgery, Washington University, School of Medicine, St. Louis.

In peripheral mammalian nerve, differential depression may be produced by strong tetanic shocks from an induction coil, which at proper strength block conduction in all myelinated fibers and allow all non-myelinated fibers still to conduct. The mammalian vagus nerve may be arranged, still attached peripherally to the body, on 3 sets of electrodes; stimulating electrodes next the body, a pair of lead electrodes at the cut central end, and a pair of blocking electrodes between. The ascending action currents due to respiratory inspiration, recorded at 500 mm./mv. amplification, are 5 to 10 mm. in amplitude (cat). These may be blocked temporarily and reversibly, to record base-line noise. The responses may be rendered monophasic by application of 1% cocaine to the killed end of the nerve.

Single strong shocks are then applied. If the myelinated fibers are blocked, only single C waves are recorded. If they are not blocked, each stimulus is usually followed not only by the A, B, and C waves characteristic of this nerve's normal action potential, but by a further random discharge that may last for several tenths of a second. These discharges resemble the respiratory discharges in form of the individual waves, which appear to be too brief to be assignable to non-myelinated fibers. Respiratory rate or depth does not change. The effects of successive shocks are summed, until after 5 or 10, at ⅛ second intervals, the discharge may last several seconds, and become greater in amplitude and in apparent frequency than the respiratory discharge itself. In some cases shocks slightly above the maximal for the C wave block the nerve at the stimulating electrode to the extent that for many seconds no respiratory discharges can be recorded, but this random discharge following each shock persists. In one nerve it has resulted from shocks too weak to stimulate non-myelinated fibers. It must, therefore, be due to myelinated fibers. These random discharges fail as the nerve deteriorates before the failure of the typical volley action current.

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It is of interest to know whether these responses are assignable only to the larger more irritable fibers of the nerve, such as pass through the recurrent laryngeal to skeletal muscle, or whether fibers of the size range of pain fibers may be involved. The afferent fibers from the lungs and other viscera below the recurrent laryngeal are nearly all of smaller size, from 8 to 9 mm. down. After cutting between the jugular and nodose ganglia, the fibers of larger size are eliminated by degeneration, leaving at the level of stimulation as arranged above only fibers of smaller size. Shocks sufficiently strong to stimulate the non-myelinated fibers of such nerves also produce a persistent random discharge in the myelinated fibers remaining, greater in intensity than the respiratory discharges.

Fibers of the pain fiber size range are therefore capable of being stimulated to a persistent repetitive after discharge of long duration, by shocks of strength necessary to stimulate non-myelinated fibers. Experimental procedures in which both myelinated and non-myelinated fibers are caused to respond are therefore equivocal, in that the addition of non-myelinated fiber responses as the strength of shock is increased may be accompanied by an unpredictable increase in the responses of myelinated fibers. These responses, while large in total number, are so low in amplitude as to be unnoticeable at the amplification usually employed to record C wave responses.

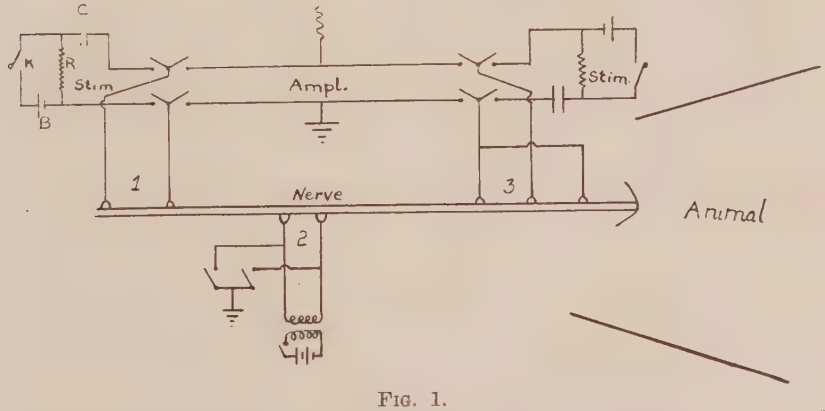

FIG. 1.

Arrangement of nerve and apparatus for differentiating between responses of myelinated and non-myelinated fibers. 1 and 3, stimulating and lead electrodes, interchangeable by double-throw switches above. 2, blocking electrodes, 3 volts on primary of coil connected for faradization, secondary set at about 6 for nerve of usual size of cat vagus or saphenous. The nerve may be blocked next to body to eliminate respiratory waves of vagus, and stimulated at middle point, by exchanging the leads from 2 and 3. C, variable condenser whose charge stimulates, B, tapped battery, R, high resistance for slow discharge of condenser between stimuli, K, key on rotating interruptor.

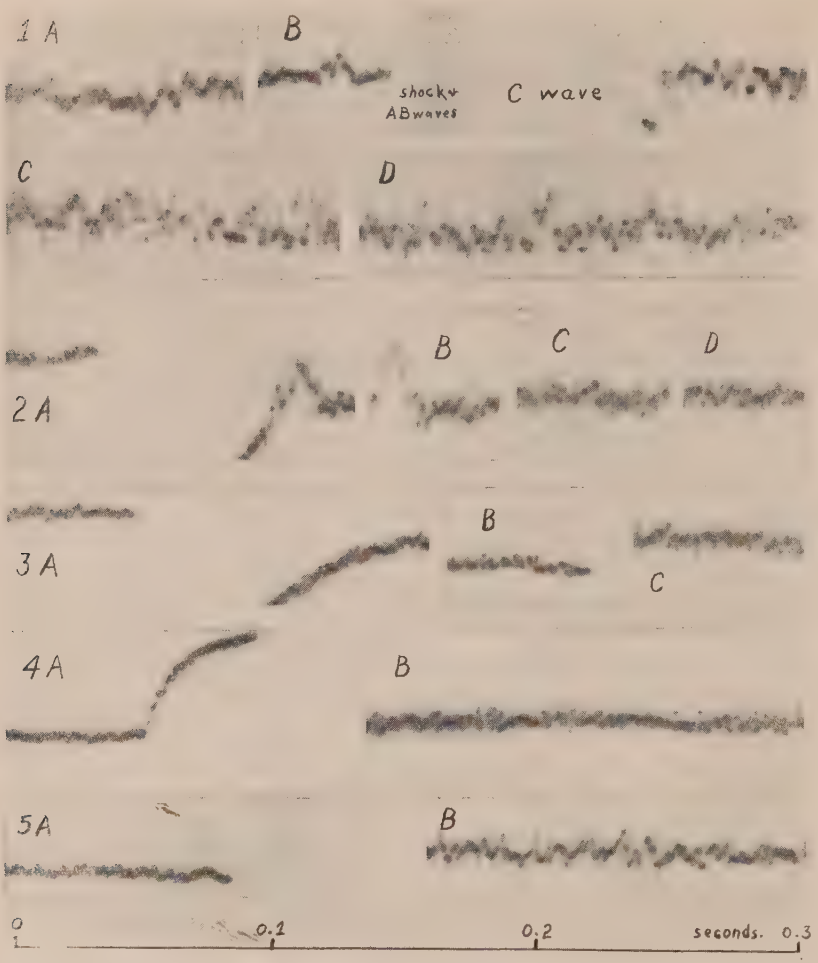

FIG. 2.

Random discharges in myelinated fibers following strong shocks to the cat vagus. 1-3, 500 mm./mv. 4-5, 350 mm./mv., reduced about ½ in reproduction.

1. A, respiratory record, maximum during inspiration. B, stimulus of just maximal strength for non-myelinated fibers, delivered toward end of inspiration. C, 0.375 seconds after a second shock, which fell 0.125 seconds after the shock of B. D, 1.25 seconds after second shock. Nerve functionally attached to body. The A, B, and C waves immediately following the shock pass far off the record.

2. Respiratory waves blocked next to body. A, first of 8 shocks of C strength. Elevation recorded is negative after potential of C wave. B, after fifth shock at 0.125 intervals. C, after eighth shock. D, one second after eighth shock.

3. An unusually sensitive nerve, shocks just below C strength. A, first shock. B, sixth shock. C, after tenth.

4. Induction coil faradization at strength sufficient to block myelinated fibers, ½ second duration. A, the first deflection is opening of ground switch, followed by stimulation. B, ½ second later. The nerve had been crushed next to body to eliminate the respiratory record.

5. A, start of faradization as above. B, 1.5 seconds later. The nerve had been cut from the body. 4A and 5A show base line noise level with and without the animal in electrical connection with the nerve. Cathode ray oscillograph, continuous strip records on bromide paper, 0.6 meters per second.

8061

Rôle of Hog Cholera Virus in Production of Inclusions in the Conjunctival Epithelium.

MARION C. MORRIS. (Introduced by L. A. Julianelle.)

*From Washington University School of Medicine.**

The occurrence of inclusions in the conjunctival epithelial cells during trachoma has been recognized and reported universally by a number of workers.[1–4, 7, 10] Whether the inclusions, however, comprise the etiological agent, as claimed in numerous publications,[5–8] or whether they are merely an incidental concomitant of the disease remains to be determined. One of the main causes of confusion regarding the rôle of the inclusion body in trachoma has been the presence of identical epithelial inclusions in other ocular infections; as inclusion blennorrhea, swimming bath conjunctivitis, vernal catarrh, etc., and even in the genito-urinary tract of man.

In accord with most investigators who have studied experimental trachoma, reports from this laboratory indicate that the inclusion does not occur in monkeys experimentally infected with the disease.[9, 11] The question arises, therefore, whether it is possible to stimulate epithelial inclusions of the conjunctiva in animals. Uhlenhuth and Boeing[12] reported that inclusions similar to those found in trachoma are present in the conjunctival epithelial cells of hogs suffering from hog-cholera. Halberstädter, who with Prowaczek was co-discoverer of the trachoma inclusion, examined Uhlenhuth's preparations and agreed to their resemblance to trachoma inclusions.[13] Since such a high percentage of animals with hog cholera has been reported to have inclusions in the conjunctival epithelium (Uhlen-

* Conducted under a grant from the Commonwealth Fund of New York City.

1 Halberstädter, L., and Prowaczek, S. von, *Deutsch. med. Woch.*, 1907, **33**, 1285.

2 Axenfeld, T., *Die Aetiologie des Trachoms*, Gustav Fischer, Jena, 1914.

3 Taborisky, J., *Arch. f. Ophth.*, 1930, **124**, 455.

4 Lumbroso, V., *Arch. Inst. Past. de Tunis*, 1924, **13**, 203.

5 Lindner, K., *Arch. f. Ophth.*, 1911, **78**, 345.

6 Rötth, *Arch. f. Ophth.*, 1932, **128**, 381.

7 Thygeson, P., *Arch. Ophth.*, 1934, **12**, 307.

8 Thygeson, P., *Am. J. Ophth.*, 1933, **17**, 1019.

9 Stewart, F. H., *Eighth Annual Report of the Giza Memorial Ophthalmic Laboratory*, Cairo, 1933, p. 113.

10 Julianelle, L. A., and Harrison, R. W., *Am. J. Ophth.*, 1934, **17**, 1035.

11 Julianelle, L. A., and Harrison, R. W., *Am. J. Ophth.*, 1935, **18**, 10.

12 Uhlenhuth and Boeing, *Berl. Klin. Wch.*, 1910, **47**, 1514.

13 Halberstädter, L., *Berl. Klin. Wch.*, 1910, **47**, 1515.

huth, *et al.*, 88-100%,[12, 14] Himmelberger 95%[15]), it seemed possible to study the nature of the inclusion body by means of the hog-cholera virus.

For this reason, hog-cholera virus obtained through the courtesy of Dr. O. S. Crisler of the University of Missouri was inoculated into hogs by various routes: subconjunctival, subcutaneous, intraperitoneal, as well as by intravenous injection, and conjunctival swabbing. All of the animals inoculated exhibited the typical symptoms of the disease including conjunctivitis. Eventually the animals died or were sacrificed, when *in extremis*. While conjunctivitis is one of the commonly reported symptoms of hog-cholera, the presence of numerous bacteria in the conjunctiva of the animals infected experimentally made it doubtful whether the eye infection was due to hog-cholera virus or merely to secondary bacterial invaders. Scrapings of the conjunctiva were made daily for a period of about 2 weeks, stained by Giemsa, and examined for inclusions. In none of the animals were inclusions found. Both before and after inoculation of virus, one hog showed numerous epithelial cells containing bodies, which were quite large, round masses, apparently comprised of tiny rod-like elements, although sometimes appearing quite homogeneous. They were usually stained a deep blue by Giemsa but were often a paler blue or a deep pink. Frequently large numbers were massed into one epithelial cell and they were never observed extracellularly. It has not been possible to identify these bodies, but it may be reasonably assumed that they are unrelated to hog-cholera since they were present before infection. The important point is that, in any case, they did not resemble the inclusions of trachoma.

Guinea pigs, rabbits, and monkeys were inoculated in the conjunctiva by swabbing and by subconjunctival injection of the virus. Scrapings of the conjunctiva were made daily for one to 2 weeks. No signs of infection occurred and no inclusions were found.

In the experiments reported here, then, no trachoma-like inclusions were found in scrapings of the conjunctiva of hogs, guinea pigs, rabbits, or monkeys inoculated with active hog-cholera virus. If such an inclusion body occurs at all in hogs suffering from hog-cholera, it appears to be present only rarely, thus limiting its diagnostic importance. As a means of studying the nature of the trachoma inclusion, however, infection of animals with hog-cholera virus is inadequate.

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[14] Uhlenhuth, Haendel, Gildemeister, *Schern. Arb. aus. dem. Kais. Gesundheitsamte*, 1914, **47**, 145.

[15] Himmelberger, L. R., *Am. Vet. Med. Ass. J.*, 1916, **1**, 450.

8062 P

Nature of Paralysis Produced in Amblystoma by Triturus Transplants.*

V. C. TWITTY. (Introduced by D. M. Whitaker.)

From Stanford University, California.

As previously described,[1] embryonic tissue of the salamander, *Triturus torosus*, when transplanted to Amblystoma embryos, has the peculiar effect of paralyzing the hosts over a prolonged period of time, rendering them entirely insensitive without impeding in any way their normal development, or their growth subsequent to recovery. Aqueous extracts of ground Triturus embryos produce a similar paralysis, of shorter duration, when injected into larvae or adults of Amblystoma. The paralyzing agent was also found present in many cases in adult Triturus female blood, and the suggestion was made that its presence there might be correlated with periods during which it was being deposited in the developing ova. A variety of other experiments performed in the analysis of this phenomenon are summarized in the article cited above. (See also Twitty and Elliott, *J. Exp. Zool.*, Vol. 68.)

Until recently there was little information as to the nature of the effect exerted by the substance in question, but the transplantation experiments outlined below offer strong evidence that its action is specific for the nervous system. Briefly, the method of demonstration consisted of bringing nervous and muscular tissue of *Triturus torosus* and *Amblystoma punctatum* into conjunction with one another in crucial combinations designed to reveal which of these 2 major components of the reaction system was affected by the paralyzing agent.

Experiment No. 1 (control). An inclusive piece from the back of the embryo, consisting of skin and the subjacent neural tube and muscle segments of both sides, was grafted as a unit from the tail-bud stage of Triturus to a position well ventral on the flank of an Amblystoma embryo. The graft developed independently *in situ*, and upon the subsequent differentiation of its segmental nerves and muscles contracted readily and vigorously when stimulated. During this period, however, the Amblystoma host was completely paralyzed.

Experiment 2 (control). In this experiment, the reciprocal of

* Part of investigations supported by a grant from the Rockefeller Foundation.

1 Twitty and Johnson, *Science*, **80**, 2064.

1, the same type of transplant was made in the opposite direction, from Amblystoma to Triturus. Here, as was to be expected, the Triturus host was active while the graft was paralyzed, even after its muscles were well differentiated.

Experiment 3. This experiment is a modification of 2 and the transplantation was performed in 2 steps: (a) A section of neural tube about 4 somites long was removed from Amblystoma and replaced by a corresponding length of tube from Triturus. After the graft had healed, the second step (b) was performed. A piece was removed from the back of the host, but now included not only the original graft of Triturus cord, but also the adjacent somites of the Amblystoma host. This graft was then placed as in (1) and (2) on the side of a Triturus embryo.

This experiment is to be compared with that in (2). As in the latter, muscles of Amblystoma are now developing on Triturus. There is the difference, however, that these Amblystoma muscles are supplied with Triturus nerves. Knowing that the muscles are exposed to a large dosage of the paralyzing agent from the host, one is in position to determine whether they are immobilized as in (2), where the innervation was from Amblystoma cord, *i. e.*, from cord of their own species.

The results were very clear. When stimulated, the Amblystoma muscles responded by strong contractions. It would seem that the conclusion to be drawn from this result is that the paralyzing agent of Triturus, in exerting its effect on Amblystoma, operates specifically on the nervous system, thereby affecting the muscles only indirectly. The power of the latter to contract is not impaired.

Experiment 4. This experiment is the reciprocal of 3. By an operation in 2 steps, as before, Triturus muscles were transferred to Amblystoma, where they developed in conjunction with a segment of Amblystoma nerve tube. The Amblystoma host was paralyzed by the Triturus tissue, as in (1). In contrast to (1), however, the grafted Triturus muscles did not contract. They are rendered immobile by their own action on the Amblystoma nerves supplying them.

8063 P

Effect of Repeated Washing on Stimulation of Yeast Respiration by 2-4 Dinitrophenol.*

J. FIELD, 2nd, AND A. W. MARTIN

From the Department of Physiology, Stanford University.

The stimulation of yeast respiration by 2-4 dinitrophenol (DNP) observed by Plantefol,[1] Field, Martin and Field,[2, 3] and others has been ascribed by Genevois and Creac'h[4] to the presence of extracellular catalysts carried over from the growth medium. The latter authors attribute the failure of Genevois and Saric[5] to note such stimulation to the fact that their yeast was always carefully washed before respiration was measured.

This suggestion seemed improbable to us, since it has been routine practice in our laboratory to wash yeast one or more times by centrifugation before measurement of respiration.[3] However, we have repeated the determination of the optimal concentration of DNP at pH 6.8, washing up to 20 times by centrifugation at 3000 r.p.m. for 5 minute periods either in distilled water or in 0.1 M phosphate buffer pH 6.8 before each experiment. The yeast was carefully resuspended between each washing. Two strains of yeast were used, our own pure culture of *Saccharomyces cerevisiae*,[2, 3] and a pure culture isolated from French baker's yeast supplied by Genevois, whose kindness we wish to acknowledge. The further experimental procedure has been described elsewhere.[3] Our results are presented in Table I.

It is clearly shown in the table that stimulation of yeast respiration by DNP is not abolished by washing either in distilled water or in phosphate buffer, once or 20 times. While the absolute values of the respiratory rate are low after 10 washings in distilled water, viability determinations by the method of Fink and Kühles[6] showed

* Supported by grants from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine and from the Committee on Scientific Research of the American Medical Association.

1 Plantefol, L., *Compt. Rend. Soc. de Biol.*, 1933, **113**, 147.

2 Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

3 Field, J., 2nd, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, 1934, **4**, 405.

4 Genevois, L., and Creac'h, P., *Compt. Rend. Soc. de Biol.*, in press.

5 Genevois, L., and Saric, R., *Compt. Rend. Soc. de Biol.*, 1932, **111**, 181.

6 Fink, H., and Kühles, R., *Z. physiol. Chem.*, 1933, **218**, 65.

TABLE I.
The Effect of Washing on the Stimulation of Yeast Respiration by DNP.
Each series, 6 experiments, 24 readings.

Concentration sodium dinitrophenoxide (mg.%)	0	30	40	50
Concentration undissociated DNP. Millimols × 10–3	0	2.22	2.96	3.70
Part 1. *Saccharomyces cerevisiae*				
Yeast washed 10 times in dist. water.				
†Suspension medium, glucose phosphate.				
Rate of oxygen consumption (cont. = 100)	100	171	194	150
Yeast washed 20 times in phosphate‡				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	103	122	81
Part 2. French baker's yeast (Genevois).				
Yeast washed once in phosphate.‡				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	161	165	156
Yeast washed 20 times in phosphate.‡				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	155	161	159
Yeast washed once in distilled water.				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	108	138	116
Yeast washed 10 times in dist. water.				
†Suspension medium, glucose phosphate.				
Rate of O consumption (cont. = 100)	100	118	143	127

*Suspension medium refers to the medium in which the yeast was suspended during the measurement of respiration in the Warburg apparatus. In every case this was 1% glucose, 0.1 M phosphate buffer, pH 6.8.

‡Phosphate = 0.1 M phosphate buffer, pH 6.8.

that this is not due to decrease in the number of living cells, and the values of percentage stimulation are in line with the rest. Accordingly we conclude that DNP acts directly on systems within the yeast cell, and that extracellular catalysts are not essential for such action. This is in accord with the view of Plantefol[7] and our own earlier opinion.[3]

[7] Plantefol, L., *Compt. Rend. Soc. de Biol.*, 1934, **117**, 1167.

8064 C

Effect of Amytal upon Pilocarpine-Induced Submaxillary and Gastric Secretion.*

MARY F. MONTGOMERY. (Introduced by C. D. Leake.)

From the Department of Surgery of the University of California Medical School, San Francisco.

The only note on the action of amytal upon the secretory response of the submaxillary gland to pilocarpine is that of Stavraky,[1] who observed that even after doses of amytal large enough to abolish the effects of stimulation of the chorda tympani and injections of acetyl choline the submaxillary gland still secreted vigorously in response to injections of pilocarpine.

My experiments deal with both the immediate and delayed effects of amytal upon the secretion of submaxillary saliva and of gastric juice induced by pilocarpine. Three dogs having permanent submaxillary fistulae were used. Observations were made upon 2 normally innervated glands (Dogs 22 and 457) and upon 2 glands after the chorda tympani nerves had been cut at the point where they separated from the lingual nerves (Dogs 22 and 205). One of the dogs also had a denervated gastric pouch which included the midportion of the greater curvature.

The pilocarpine (0.2, 0.4 and infrequently 0.6 mg. per kg.) was given intravenously to eliminate the factor of irregular absorption. With rare exceptions pilocarpine was not injected more frequently than once every 24 hours. Secretion began within 20 seconds after the injection. Collections were made every 5 minutes from the appearance of the first drop to the practical cessation of the secretion. Under these conditions the quantity of saliva secreted by individual animals on successive days followed curves of fairly constant form, height and duration, and there appeared to be no change in the sensitivity of the animals to pilocarpine after repeated injections. When the normal influence of pilocarpine upon the gland had been determined, amytal was administered either orally or intravenously as the sodium salt in doses of from 5 to 65 mg. per kg. Immediately after a 45 mg. per kg. dose there was little if any change in the character or duration of the effect of pilocarpine. However, at 6 hours, when the animal had not entirely recovered consciousness,

* This work has been conducted under a grant from the Christine Breon Fund for Medical Research of the University of California Medical School.

1 Stavraky, G. W., *J. Pharm. and Exp. Therap.*, 1931, **43**, 499.

TABLE I.
Summary of Experimental Results.

Dog	Structure	Dose mg. per kg. Pilocarpine I.V.	Na Amytal I.V.	Amytal Orally	Duration of Pilocarpine —Response in Min.— Normal	2nd day after amytal	Return to Normal Days
22	Normal submaxillary	.4	45		115-130	50	11
	Submaxillary—Chorda tympani cut 6 days before	.4	40		115	60	6
	Submaxillary—Chorda tympani cut 12 days before	.4		5	115	110	
457	Normal submaxillary	.6	45		160	60	
	,, ,,	.6	55		160	45	13
	,, ,,	.6	65		155-170	70	13
	,, ,,	.2	45		100	40	9
	,, ,,	.2		20	110	60	8
	,, ,,	.2	10		105	55	6
	,, ,,	.2	5		95	90	
205	,, ,,	.2	20		90	40	
	Submaxillary—Chorda tympani cut 19 days before	.2		20	80	50	More than 6
	Denervated gastric pouch	.2		20	85-90	50	More than 6

pilocarpine stimulated a definitely smaller flow of saliva although the reaction was normal in duration. At 18 hours, while the dog was still slightly ataxic, the secretory response to pilocarpine was still depressed and moreover was strikingly shortened. From this time on there was a progressive diminution in the duration of secretion to a minimum on the second to third day. At the same time the secretory response of the gland in the first 5 minutes after the injection of pilocarpine was normal or even greater than the control. After the third day there was a gradual return toward the normal duration, but in this experiment the recovery was not yet complete on the ninth day after the injection of the amytal.

Protocol: Dog 457; wt. 11.6 kg.; right submaxillary fistula.

Dose of pilocarpine 0.2 mg. per kg.

Control: Secretion in first 5 min. 5.06 cc. Duration of the effect of pilocarpine 85 minutes. Total quantity of saliva produced 23.45 cc.

On the following day the dog received 45 mg. per kg. of sodium amytal intravenously.

6 hrs. after giving sodium amytal: Dog has not entirely recovered from the anesthesia. Secretion in first 5 min. 2.82 cc. Duration of secretion 85 minutes. Total quantity of saliva 15.94 cc.

At 18 hrs.: Dog slightly ataxic. Secretion in first 5 min. 3.33 cc. Duration 60 min. Total quantity 10.58 cc.

At 25 hrs.: Dog fully recovered. Secretion in first 5 min. 4.82 cc. Duration 40 min. Total quantity 9.99 cc.

At 44 hrs.: Secretion in first 5 min. 5.30 cc. Duration 40 min. Total quantity 9.01 cc.

At 66 hrs.: Secretion in first 5 min. 5.30 cc. Duration 40 min. Total quantity 11.94 cc.

6th day: Secretion in first 5 min. 6.54 cc. Duration 70 min. Total quantity 16.46 cc.

9th day: Secretion in first 5 min. 5.84 cc. Duration 75 min. Total quantity 15.94 cc.

The effect described is independent of the influence of the parasympathetic centers since it occurred to the same degree before and after the chorda tympani had been cut and since it also was observed in the case of a denervated gastric pouch. Neither is the effect one of simple dehydration, for although the withdrawal of water for 48 hours diminished the amount of saliva produced by Dog 22 in response to injections of pilocarpine, this change was a simple diminution in volume without shortening of the duration of the response.

Table I gives a summary of the experiments showing the doses of pilocarpine and amytal used, the degree of shortening of the secretory effect of pilocarpine and the time required for return to normal. It will be noted that amytal administered by either the oral or intra-

venous route is effective and that all doses above 10 mg. per kg. produce a delayed shortening of the effect of pilocarpine on the second day. The amount of shortening and particularly the duration of the recovery period are related to the dose but are not definitely proportional to it.

Doses of 5 mg. per kg. of amytal by mouth did not significantly alter the response of the denervated salivary gland of Dog 22 to pilocarpine. However, when this same dose was repeated on 4 successive days a very definite shortening of the duration of the effect of pilocarpine was evident on the fifth day. This result taken in conjunction with the long duration of the recovery period after large doses of amytal indicates that sodium amytal or its decomposition products may remain in the tissues for a considerable length of time.

8065 P

A New Tick Vector of Relapsing Fever in California.

C. M. WHEELER, W. B. HERMS, AND K. F. MEYER.

From the Division of Entomology and Parasitology, College of Agriculture, and the G. W. Hooper Foundation, University of California, Berkeley, and San Francisco, California.

Relapsing fever has been repeatedly reported in California since 1921 (Briggs,[1] Porter, Beck and Stevens,[2] and Coleman[3]). While ticks have been suspected as vectors for some time (Herms and Wheeler,[4] and Coleman[5]), no positive experimental evidence incriminating a particular species has been obtained until recently.

A careful search for ticks has been made repeatedly in various localities in California where cases of relapsing fever had been reported. On August 12, 1931, three specimens of mature *Ornithodorus* ticks were taken in a cottage located at Brockway, Lake Tahoe, California, at an elevation of approximately 6,000 feet, where cases of this disease had occurred about a month previously.

1 Briggs, *J. Am. Med. Assoc.*, 1922, **79**, 941.

2 Porter, Beck, and Stevens, *Am. J. Public Health*, 1932, **22**, 1136.

3 Coleman, *J. Infect. Dis.*, 1933, **53**, 337; and 1934, **54**, 11.

4 Herms and Wheeler, paper presented at a joint meeting of Section N (Medical Sciences), A.A.A.S., and the American Society of Parasitologists, June 20, 1934, in Berkeley, Calif. Manuscript.

5 Coleman, *J. Infect. Dis.*, 1934, **54**, 281.

On examination and comparison with other species of the genus *Ornithodorus* these specimens were believed to be an undescribed species. In September, 1933, several similar specimens of *Ornithodorus* were sent to us from Big Bear Lake, California. In August, 1934, more ticks of the same undescribed species were collected in a relapsing fever cabin at Big Bear Lake in San Bernardino County, elevation about 5,700 feet. Other specimens of this species have been taken in various localities in the following counties: San Bernardino, Eldorado, and Placer, at elevations ranging from 5,000 to 8,000 feet.

Since Beck and her associates[2] had demonstrated spirochaetes in the blood and organs of squirrels and chipmunks both at Big Bear Lake and at Lake Tahoe and thus established the burrowing rodents as possible intermediate hosts, collections of rodent nesting material taken from the cabins were examined thoroughly. Several dozen specimens of ticks of the same species were discovered. Specimens of ticks similarly collected have also been obtained through the courtesy of Dr. E. B. Godfrey, San Bernardino County Health Officer.

The first transmission experiment was conducted at the Hooper Foundation in San Francisco, California. Six specimens of *Ornithodorus* n. sp. which had been taken at Eagle Point, Big Bear Lake, California, were placed on the shaven abdomen of a Macacairus monkey, No. 1038, on September 23, 1934. The ticks were allowed to engorge to repletion and detached of their own accord (19 to 31 minutes). At the site of the bite a bright hemorrhagic area (3 x 5 mm.) appeared and persisted for 48 hours. Sixteen days later, on October 9, 1934, this monkey showed a characteristic rise in temperature and spirochaetes were demonstrated in a blood smear. Subsequent subinoculations into white mice also produced the spirochaetes in the blood of these animals. On October 23, or 14 days later, the monkey had a relapse with a few spirochaetes in the blood.

The next series of transmissions were carried on in the Division of Entomology and Parasitology, University of California. Five specimens of *Ornithodorus* n. sp. in varying stages of development, taken at Big Bear Lake, California, were placed on the abdomen of a series of 4 white mice, and were allowed to engorge (average time of feeding 26 minutes). Four to 7 days later spirochaetes were demonstrated in blood smears. No coxal fluid or fecal material was observed to exude from the ticks while feeding. Subsequent inoculations into other white mice also produced spirochaetes in the blood of these animals.

Ornithodorus hermsi, the proposed name for the new species, differs markedly from the 4 species of *Ornithodorus* reported from California, namely: from *O. turicata* in (a) the absence of clubbed hairs between the mammillae, (b) the coxae I narrowly separated from coxae II, (c) the arrangement and number of teeth on the hypostome, (d) the arrangement and number of protuberances on tarsi I and IV in particular, and (e) the smaller size of the female. This new species differs from *O. talaje* in (a) the absence of large discs on the dorsum, (b) the characteristic sculpturing of the integument, (c) microscopical differences in the structure of the integument, (d) the arrangement of the dentition of the hypostome, (e) the absence of lateral flap-like borders at the margins of the capitulum, (f) the cheliceral teeth, (g) the shape of the anal grooves, and (h) the tarsi of the legs bearing diagnostic protuberances. To those familiar with the other 2 California species, *O. coriaceus* and *O. megnini,* differences need not be listed.

Brumpt[6] has recently designated the spirochaete responsible for the human cases of relapsing fever in Texas and California as *Spirochaeta turicatae* n. sp. Until the susceptibility of the California ticks and their ability to act as vectors for the Texan spirochaete has been experimentally proven and, *vice versa,* the vector ability of *Ornithodorus turicata* for the California *Spirochaeta recurrentis,* it is doubtless premature to consider the identity of the 2 parasites as established. In fact, it is not unlikely that the Colorado[7] and British Columbia[8] relapsing fever infections are transmitted by ticks which differ from the *O. turicata* and *O. hermsi.* Investigations along these lines are in progress.

[6] Brumpt, *C. R. Soc. Biologique,* 1933, **113**, 1369.

[7] Meader, C. N., *U. S. Public Health Rep.,* 1915, **30**, No. 52, 3737; Waring, T. T., *Am. J. Med. Sci.,* 1918, **155**, 819.

[8] Palmer, T. H., and Crawford, D. J. M., *Canadian M. A. J.,* 1933, **28**, 643; Hearle, E., *Canadian M. A. J.,* 1934, **30**, 494.

8066 C

Organ-Forming Areas in the Early Chick Blastoderm.

B. H. WILLIER AND MARY E. RAWLES.

From the Department of Zoology, University of Rochester.

In attempting to locate the various organ-forming areas of the early blastoderm, a series of transverse and longitudinal cuts has been made at certain measured distances from the primitive pit in such a manner as to divide a single blastoderm of the head-process stage of development into a definite number (either 11 or 18) of median and lateral pieces. The developmental potency of each piece was tested in the chorio-allantoic membrane. Each part of the blastodisc is found thereby to have a certain capacity for development or specific organization, which becomes expressed in the form of specific tissues. Nearly 400 grafts have been examined histologically, a generalized analysis of which follows.

1. *Differences in developmental capacity along the antero-posterior axis.*[1] The portion of the area pellucida anterior to the head-process and that part of the area containing the posterior half of the primitive streak yield grafts much less frequently and invariably smaller ones than do those portions including either the node or the head-process. Also, the grafts from these portions exhibit a similar difference in the quantity of organ tissues produced, the node and process portions showing by far the greater variety and amount of tissues. Very striking is the demonstration that particular structures develop only from rather definite regions or areas of the blastoderm. So characteristic are some organ tissues of particular regions that we can speak of eye-forming, thyroid-forming, ear-forming, or mesonephros-forming levels or areas. The eye and thyroid-forming areas are associated with the anterior end of the head-process, ear farther posteriorly at the node level, while the center of the mesonephric area lies just posterior to the primitive pit (Fig. 1).

2. *Differences in developmental capacity along the medio-lateral axis.* Medio-lateral differences in the ability of the transplant to survive and grow as a graft are very noticeable. Graft frequencies are consistently higher for the median pieces, distinctly lower for the left, and lowest for the right pieces. Mesonephros develops, for example, with a frequency of 70% from median pieces, 18% and 11% from left and right pieces respectively. Also at the brain-

[1] Rawles and Willier, *Anat. Rec.*, 1934, **58**, (Sup.), 34.

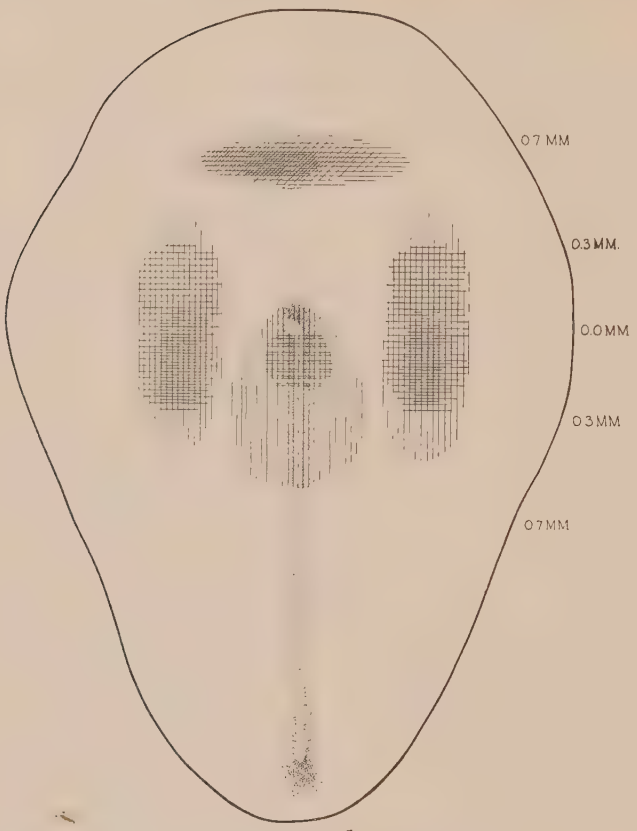


FIG. 1.

Map showing the position and shape of the eye-, heart-, and mesonephros-forming areas of a blastoderm of the head-process stage. The density of shading within areas indicates roughly the intensity of developmental potency. Only the region of greatest intensity of mesonephros formation, localized just behind the pit, has the power to form adrenal and gonad. X ca. 37.

forming levels nervous tissue illustrates very nicely the same kind of frequency differences, occurring in 100% of the median grafts, 70% of left and 50% of right. Further, differences in the size of grafts and the amount of recognizable differentiated tissue obtained from median and lateral pieces closely parallels the frequency differences.

Distinct medio-lateral gradations are evident as regards degree of differentiation attained by various structures. The eye, for example, which differentiates from a median piece may show all the constituent parts—retina, pigmented layer, lens—while grafts from lateral pieces seldom show more than the pigmented layer. In grafts from right pieces, particularly, a mere trace of the pigmented layer

is the sole representative of this organ. Similar conditions obtain with respect to differentiation of mesonephros where, again, in median grafts all the component elements are present and exhibit a higher grade of differentiation. Closely associated with the well-differentiated mesonephros of the median pieces, but absent in either of the laterals, are suprarenal and gonad (Fig. 1).

Many organs develop in obvious relation to medio-lateral organization: some from both median and lateral pieces, others only from median pieces, and still others only from lateral pieces. Structures occurring in both lateral and median grafts are brain parts, eye, ear, thyroid, mesonephros, skin, and the somite derivatives, cartilage, bone and muscle. Most of these structures developing in all 3 sections of a transverse level occur, as noted above, in far higher percentages in the median pieces and grade off laterally. Chorda, hypophysis, suprarenal, gonad and spleen have occurred only in median grafts. Heart, liver and feather germs seem limited to lateral areas, *i. e.*, bilaterally located. The heart-forming area is quite elongated antero-posteriorly extending from a level just behind the anterior end of the process to a level 0.3 mm. to 0.4 mm. posterior to the primitive pit. At one certain part of this elongated area (posterior node level), however, heart tissue develops with greatest frequency (90%) and quantity and from here in both respects declines rather gradually anteriorly and sharply posteriorly (Fig. 1). The capacity to produce liver, although coinciding with the heart region, apparently does not extend so far posteriorly. The frequency of its formation is definitely lower than that of heart. The medio-lateral extent of the heart-liver area has not yet been definitely ascertained.

3. *Changes in the localization of areas with advance in development.* Certain evidence has accumulated which indicates strongly that the developmental potency within the various organ-forming areas, which extend like bands across the blastoderm at first, undergoes changes as development proceeds with consequent changes in shape of the area. We have already noted that the capacity to produce heart and liver tissue is confined to lateral areas at the head-process stage. Yet at an earlier stage (primitive-streak) the median pieces as well as the lateral have the power to produce both of these organ tissues.[2] Similarly, changes in thyroid-forming capacity occur which result in its loss from the median pieces at about the 2- or 3-somite stage.[3] In the case of eye, however, the

2 Rudnick, D., *J. Exp. Zool.*, 1932, **62**, 287.

3 Rudnick, D., *J. Exp. Zool.*, 1935, in press.

median portion of the blastoderm retains its eye-forming potency even later, *i. e.,* until approximately the 8-somite stage.[4]

4. *Characteristics of the organ-forming areas.* Each organ-forming area exhibits a gradient in developmental potency, the intensity of which gradually diminishes from the center peripherally until it completely disappears. These areas are then not definitely circumscribed and furthermore, the indefinite boundaries of adjacent ones overlap one another.

Areas lying transverse to the antero-posterior axis, *i. e.*, eye and mesonephros, exhibit a distinct asymmetrical organization—the right portion showing far less developmental potency than the left. The developmental potency which is invariably the highest in the median portion, usually falls off abruptly to the right and gradually to the left of the area (Fig. 1).

Although the present experiments were not designed to limit the boundaries of the various organ-forming areas, there are nevertheless, some definite indications that the shape or contour of each area is more or less characteristic of the organ system. In general it is somewhat elongated either in a transverse direction (eye, thyroid, lung epithelia, etc.) or in an antero-posterior direction (heart, mesonephros, etc.). The eye-forming area is thus far the only one which has been carefully mapped—its outline is eliptical (Clarke).

With advance in development the gradient in developmental potency originally characteristic of the area changes (apparently rapidly in some organ-forming systems and slowly in others) with the result that its shape becomes markedly altered. There is some evidence that the ultimate shape assumed by an area, just prior to the establishment of the organ rudiment, is in accordance with the configuration of the organ system to be formed.

[4] Clarke, L. F., *Anat. Rec.*, 1934, **58** (Sup.), 54.

8067 C

Action of Acetyl Beta Methylcholin on Ventricular Rhythms Induced by Adrenalin.

M. H. NATHANSON.

From the Department of Medicine, University of Minnesota, Medical Service, General Hospital.

Fibrillation of the ventricles is the most frequent cause of sudden cardiac death. It is generally accepted that this is the mechanism of sudden death characteristic of coronary arterial disease in man. Adrenalin tends to raise the rhythmicity of the ventricular foci leading to ectopic ventricular rhythms. There is considerable evidence that accelerator nerve impulses and adrenalin play an important part in the genesis of ventricular fibrillation. Levy and Lewis[1] induced ventricular fibrillation by the injection of adrenalin in cats under light chloroform anesthesia. Otto[2] observed that section of the cardioaccelerator nerve fibers prevented the ventricular fibrillation which follows ligation of branches of the coronary circulation. Nahum and Hoff[3] found that inhalation of benzol regularly produced ventricular fibrillation. This did not occur in animals in which the adrenal glands had been removed. Hoff and Nahum[4] showed that removal of the stellate ganglia and adrenal glands enormously decreased the susceptibility of the heart to ventricular fibrillation following electric shock. They concluded that adrenalin acts synergistically with some other factor to increase ventricular rhythmicity leading to ventricular fibrillation.

Acetyl beta methylcholin, a synthetic choline derivative, produces effects similar to those which follow stimulation of the parasympathetic nerves. Nahum and Hoff[5] demonstrated that the ventricular fibrillation induced by inhalation of benzol can be prevented by adequate amounts of acetyl beta methylcholin. Hoff and Nahum[4] more recently showed that this substance lessened the susceptibility of the ventricles to fibrillation following electric shock. In the present study, ectopic ventricular rhythms were produced in 6 elderly patients by the intravenous injections of 0.1 mg. of adrenalin. Electrocardiographic records were taken before the injection and a

1 Levy, A. J., and Lewis, T., *Heart*, 1911, **3**, 99.

2 Otto, H. L., *Arch. f. d. ges. Physiol.*, 1927, **217**, 528.

3 Nahum, L. H., and Hoff, H. E., *J. Pharm. and Exp. Therap.*, 1934, **50**, 336.

4 Hoff, H. E., and Nahum, L. H., *Am. J. Physiol.*, 1935, **110**, 675.

5 Nahum, L. H., and Hoff, H. E., *Am. J. Physiol.*, 1934, **109**, 78.

continuous record following the injection until the reaction had subsided. In each case ectopic ventricular beats from multiple foci appeared within a minute, quickly increasing in number. The height of the reaction took place within the first 2 minutes and at this time practically all the beats were ectopic ventricular in origin. (Fig. 1, C.) The effect of the adrenalin subsided rather quickly so that there was a return to normal rhythm within 5 minutes after the injection. (Fig. 1, F.) Fifteen minutes after this reaction had subsided, acetyl beta methylcholin chloride, 20 mg., was injected subcutaneously. Adrenalin, 0.1 mg., was again injected intravenously and the reaction observed with a continuous electrocardiographic record.

In the first patient the second dose of adrenalin was administered intravenously one and one-half minutes after the subcutaneous injection of acetyl beta methylcholin. At this time the systemic effects such as flushing of the face, sweating and salivation were most intense and there was an increase in pulse rate. In this case, the second dose of adrenalin induced ventricular premature beats sim-

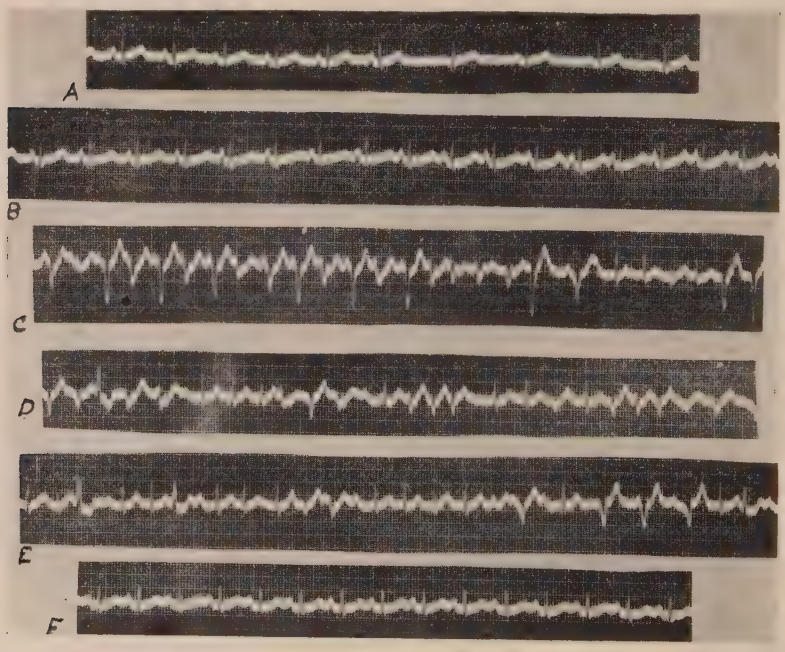


Fig. 1.

(Patient M.D.) A, lead 2, before injection of adrenalin. B, taken 30 seconds after injection of adrenalin. Lower strips taken at one-minute intervals following the injection. Note ectopic ventricular beats from multiple foci in strips C, D, and E.

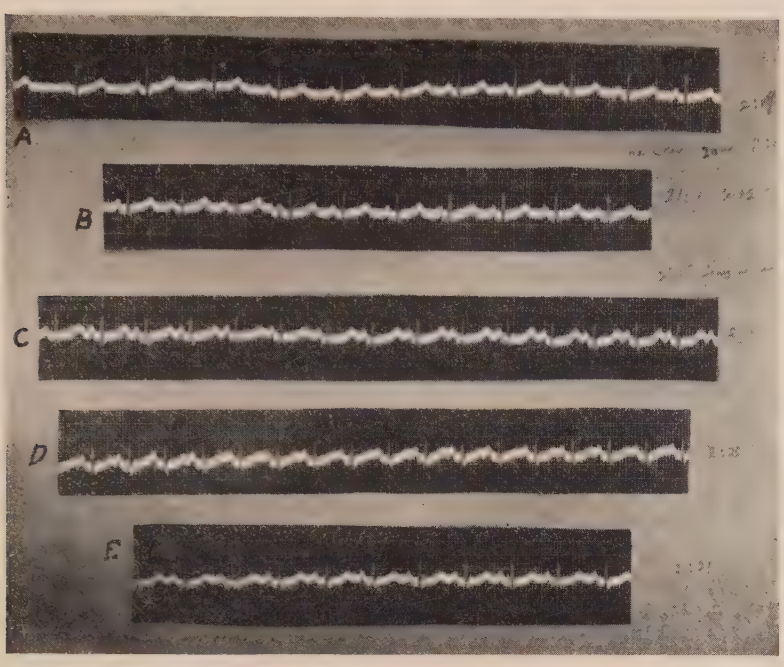


FIG. 2.

(Patient M.D.) A, lead 2, before the administration of acetyl beta methylcholin. B, taken 2 minutes after subcutaneous injection of acetyl beta methylcholin chloride, 20 mg. C, taken 2 minutes after the intravenous injection of 0.1 mg. adrenalin which was administered 6 minutes following the acetyl beta methylcholin. D and E taken 4 and 5 minutes after the adrenalin. Note complete absence of ectopic ventricular beats.

ilar to those observed with the first dose of adrenalin. In the second patient the initial rise in heart rate following the acetyl beta methylcholin changed after an interval of 5 minutes to a rate slower than that of the control period. The second dose of adrenalin was then administered 6 minutes after the acetyl beta methylcholin. There was only a slight reaction so that during the entire period of 10 minutes only 2 ventricular ectopic beats appeared. In the remaining 4 cases the second dose of adrenalin was injected 6 minutes after the acetyl beta methylcholin. In all 4 instances, adrenalin failed to induce a single ectopic ventricular beat (Fig. 2). In one case a short series of ectopic auricular beats appeared 4 minutes after the injection of the adrenalin.

Conclusion. Acetyl beta methylcholin in man tends to counteract the effect of adrenalin on the rhythmic property of the ventricles.

8068 C

A Fixed Color Standard for Cholesterol Determinations.

ARTHUR SHAPIRO, HENRY LERNER AND EDNA POSEN. (Introduced by W. M. Sperry.)

From the Richard Morton Koster Research Laboratory, Crown Heights Hospital, Brooklyn.

Schoenheimer and Sperry[1] described a micro method for free and ester cholesterol in serum, which, with the use of a photometer, gives highly accurate results. For clinical and many physiological purposes the use of a colorimeter is to be preferred. The only difficulty in applying the ordinary micro-colorimeter to the Schoenheimer and Sperry method for serum-cholesterol determinations has been the necessity for developing the color on several standard solutions of cholesterol for each series of determinations. With the kind cooperation of Dr. Schoenheimer and Dr. Sperry the problem of eliminating this obstacle was undertaken.

No changes were made in the original procedure up to the point of color development. Since it proved difficult to measure the small quantities of concentrated sulfuric acid accurately, it was found convenient to mix the acetic anhydride and sulfuric acid together. Using acetic anhydride and sulfuric acid in proportion of 15:1, it was found that with a 2 cc. sample of mixed reagent, the color reached a maximum at 27 minutes and remained constant for 10 minutes. This mixed reagent is stable for one hour only.

In seeking a fixed color standard, several substances were tried. It was found that only India Ink and Carter's Midnight Black Ink gave a true visual match. Copper sulfate and naphthol Green B were found unsatisfactory because of inexact matching. India Ink was discarded because the diluted suspension settled out on standing.

The standard solution of Carter's Ink is prepared by diluting 1 cc. to a liter with 10% acetic acid. Setting solutions of known concentration of the ink at 20 mm. on one side of the colorimeter,* and moving the standard, it was found that the length of column of the standard required to match the other solution was proportional to the concentration, thus showing that Beer's law was obeyed for a wide range of concentrations. This ink solution is standard-

[1] Schoenheimer, R., and Sperry, W. M., *J. Biol. Chem.*, 1934, **106**, 745.

* A Bausch and Lomb, Duboscq Colorimeter (60 mm.), with microcups, plungers, eye-cap diaphragm, and an Eastman Kodak No. 71A color filter in the eyepiece, was used.

ized daily against 1 cc. of a 0.0075% solution of cholesterol in glacial acetic acid.

The procedure followed is practically identical with the original one. The dried precipitate of digitonide is dissolved in 1 cc. of glacial acetic acid at 70°C. and then cooled in a water bath at 25°C. Two cc. of the mixture of anhydride and sulfuric acid is added, the solution stirred and placed in a water bath at 25° in the dark for 29 minutes. The solution is then pipetted into the cup of the micro colorimeter and set at 20 mm. The reading is obtained by matching the standard ink solution against this setting. This concentration of ink standard provides a sufficient range of intensities to cover values from less than 30 mg. free cholesterol per 100 cc. up to more than 500 mg. total cholesterol per 100 cc., by setting the unknown solution at appropriate levels.

With this procedure it has been found possible to determine known solutions of free and ester cholesterol in acetone alcohol within an average accuracy of about 4%. Only once an error of 11% was encountered when as little as 0.024 mg. of cholesterol was determined.

This method has also proved satisfactory in determining serum cholesterol. Analyses in Table I give an idea of the precision to be expected.

TABLE I.

Vol. of Serum cc.	Vol. Extract cc.	Free Chol. in 100 cc. Serum mg.	Deviations mg.	Total Chol. in 100 cc. Serum mg.	Deviations mg.
0.2	5	75	1.1	260	9
0.2	5	75.9	0.2	265	5
0.5	10	80.6	4.5	280	11
0.5	10	76.1	0	268	1
0.5	10	72.7	3.4	274	5
		Av. 76.1	1.8	Av. 269	6.2
			equivalent to 2.3%		equivalent to 2.2%

Summary. A fixed color standard for use with Schoenheimer's and Sperry's micro method for serum cholesterol is described. The use of a mixture of acetic anhydride and sulfuric acid is suggested. The method has been applied to the analysis of known solutions of cholesterol and its esters and to the determination of serum cholesterol and cholesterol esters with satisfactory results.

8069 P

Further Rabbit Heart Perfusion Experiments with Amino-Acids.

GEORGE DECHERD, GEORGE HERRMANN, AND OLVA DAVIS.

From the Department of Medicine, University of Texas, Galveston.

We have previously reported[1] our studies on the creatine content of rabbit heart muscle after perfusion of the coronary system with oxygenated Ringer-Locke solution plus glycocoll in a Dawson-Gunn-Locke apparatus. We found that a further modification of the method was necessary in order to maintain the perfusate at a satisfactory pH level. The cause of the greater part of the fall in pH was found to be the CO_2 produced by the heart's metabolism. In order to remove this absorbed and diffusible CO_2, an aerating agitating lift for the perfusate was devised with the use of a suction pump instead of oxygen pressure. The perfusate was then reoxygenated in the reservoir in the constant temperature bath just above the heart cannulae.

It was found that the creatine content of the heart muscle dropped when the pH of the perfusion fluid was allowed to fall below 7.3-7.5 (Table I). When the estimated pH of the fluid in the myocar-

TABLE I.
Influence of pH on Heart Muscle Creatine.

Perfusion Fluid pH	Creatine Mg. %
7.5	166
7.4	156
7.3	152
7.1	146
6.9	139
6.8	130

dial vessels was maintained within these limits, however, failure was postponed for several hours. Eventually there accumulated in the perfusate a non-volatile acid metabolite, lactic acid on the basis of the qualitative test, which caused an agitation-irreversible drop in the pH. In the presence of myocardial infarcts the non-volatile acid metabolites early forced down the pH and lead to a sharp loss in creatine and early failure.

We have previously[1] noted that perfusion of the isolated rabbit heart with Ringer-Locke solution, until it stopped in failure after several hours, resulted in a uniform drop in the creatine content of

[1] Herrmann, G., Decherd, G., and Erhard, P., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 547.

the heart muscle. Furthermore, we found that under similar circumstances the addition of glycocoll failed to prevent this creatine loss. This finding has been confirmed in a larger series of hearts perfused under more constant conditions made possible by the modified method described above.

In addition to the glycocoll experiments, we have studied under similarly controlled conditions the effects of several other amino-acids which have been suggested as possible factors in creatine metabolism. Among these we have thus far added to our perfusate arginine, glutamic acid, aspartic acid, methylguanidine, alanine, and creatine itself, with the results shown in Table II. In the same

TABLE II.
Rabbit Heart Muscle Creatine.

Substances used and No. of exp.	Minimum F	Minimum D	Maximum F	Maximum D	Average F	Average D
Controls	143		168		154	
10		652		831		740
Glycocoll	114		155		134	
10		562		730		645
Arginine	100		126		115	
4		462		605		545
Glutamic acid	108		134		116	
4		498		664		581
Aspartic acid	108		148		134	
4		581		682		639
Methylguanidine	126		150		138	
4		598		705		659
Creatine	125		161		138	
9		630		780		689
Alanine	136		205		166	
20		648		896		783
Insulin	111		158		130	
4		492		701		583
Infarcted Hearts	51		99		79	
14		259		531		439

Table I: Values are listed in mg. % of the fresh muscle (F) and of the dried muscle (D). Total creatinine is actually determined, but of this total less than 5 mg. % is preformed creatinine, the remainder being produced by acid hydrolysis of the muscle creatine.

table we have recorded the effects of insulin and of myocardial infarction. Alanine alone of all the substances studied seemed able to maintain and even enrich the creatine content of the heart muscle. Myocardial infarction, regardless of the perfusate used, caused a very sharp fall in the creatine values of the rabbit heart muscle.

8070 P

Creatine and Glycogen Content of Normal and Infarcted Heart Muscle of the Dog.

GEORGE HERRMANN AND GEORGE DECHERD.

From the Department of Medicine, University of Texas, Galveston.

In our studies of the effects of perfusing the rabbit hearts with Ringer-Locke solution to which various amino-acids were added,[1] we were struck by the fact that infarction of the myocardium caused a sharp drop in the pH of the perfusate. This was apparently due to the liberation of a non-volatile acid metabolite giving the qualitative test for lactic acid. The inevitable result of the drop in pH was earlier failure, and, furthermore, low heart muscle creatine values. These findings seemed to be a corollary of our previous observation[2] that myocardial infarction in the dog leads to a creatinuria.

We therefore decided to undertake chemical studies of the infarcted as compared with the normal heart muscle of the dog. In addition to the creatine content we thought it worth while to determine the glycogen content, since Riesser and Brentano, we found, had demonstrated a close relationship between creatine mobilization and glycogenolysis.[3] Himwich and Goldfarb have demonstrated a decrease in glycogen content and an increase in lactic acid in infarcted heart muscle coincident with a rise in blood lactic acid.[4]

Dogs were anesthetized with barbiturates intraperitoneally and the chest opened under artificial pressure respiration. A nick was made in the pericardium over the first part of the main anterior descending branch of the left coronary artery. The artery was isolated and tied off, and the pericardium and chest closed. The heart was then allowed to continue beating for periods varying from 15 minutes to 12 hours. The infarcted beating heart was removed and sections of infarcted and normal muscle were minced in cold alcohol for glycogen determinations; similar portions were removed for creatine and total solid determinations.

The infarcted muscle showed an immediate drop in glycogen

[1] Decherd, G., Herrmann, G., and Davis, O., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1302.

[2] Herrmann, G., and Decherd, G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 478.

[3] Riesser, O., and Brentano, C., *Arch. f. exp. path. u. pharm.*, 1930, **155**, 1.

[4] Himwich, H. E., and Goldfarb, W., *Am. J. Physiol.*, 1934, **109**, 403.

TABLE I.
Comparison of Normal and Infarcted Heart Muscle.

Duration Min.	Loss in Infarcted Muscle % Creatine	Glycogen	Gain in Water Content in Infarcted Muscle
18	0	56	0.0
20	4	22	0.66
30	2	21	1.60
120	5	52	2.10
200	2	25	0.54
315	38	87	3.98
340	43	76	3.08
420	32	65	2.55
450	20	19	4.14
690	52	70	3.38
720	40	83	2.70

content as compared with the normal muscle (Table I). The drop seemed to have reached its maximum in 5 hours. Edema detectable by a drop in total solids apparently began to appear in the infarcted anoxic muscle within a half hour and likewise reached its maximum in 5 hours. The creatine loss was slight up to 5 hours, after which diffusion from the damaged muscle was considerable, and increased slowly.

8071 C

Reactions of Ant. Pituitaries of Male Rats to Administration of Ant. Pituitary-Like Substance and to Oestrin.*

J. M. WOLFE AND DORIS PHELPS.

From the Departments of Anatomy and Gynecology, Vanderbilt University School of Medicine, Nashville, Tennessee.

Recent studies[1] indicated that injections of massive amounts of oestrin into normal female rats induced weight and morphologic reactions in the anterior pituitary similar to those obtained by the injection of the anterior pituitary-like substance of pregnancy urine. Both of these substances induced a marked weight increase in the gland, a marked loss of granules from the basophiles and a less evident loss of granules from the eosinophiles (in A.P.L. rats whose ovaries contained active corpora lutea). Furthermore, it has been found that administration of the A. P. L. factor has no action on

* These studies were aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

1 Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1192.

the anterior hypophysis of the castrated rat[2, 3] but that oestrin is capable of direct action on the anterior lobe of the castrated rat.[4] The experiments described below were carried out in order to compare the reaction of normal male rat pituitaries to administration of these 2 factors.

Fourteen adult male rats received daily injections of 25 rat units of an extract of pregnancy urine† for 10 days. Another group of 10 rats received daily injections of 200 rat units of oestrin‡ for 10 days. A third group of 30 normal mature male rats served as controls. At autopsy, body, pituitary, testes and prostate and seminal vesicle weights were secured. Serial sections of all glands were cut, 5 representative sections from each series were studied and cell counts made. Quantitative results are presented in Table I.

Administration of the A. P. L. substance to male rats did not increase the weight of the pituitary gland over that found in the controls (Table I). This was in contrast to previous findings in female rats. Confirming the findings of Severinghaus[2] the anterior pituitaries of the injected male rats exhibited a marked loss of granules from practically all the basophiles although a few granular basophiles were present (Table I). We have previously reported that a few typical castration cells were found in the anterior lobes of normal male rats. These were also found in the control males used in this series. Whether such cells are found in the anterior lobes of male rats generally or whether this condition is characteristic only of our colony is unknown at present. In the male rats receiving the A. P. L. substance, an occasional castration cell was observed. They were never abundant enough, however, to be presented in terms of percentage. There were no significant changes in the eosinophiles or the chromophobes. Table I shows that these cells were found at practically the same level in the A. P. L. injected and the control rats. In all sections studied, the total number of mitoses were counted and are expressed in Table I as the mean number per section. Mitoses were of equal number in the male rats receiving A. P. L. and in the controls (Table I). Cell counts were made on every fifth field of the sections studied. Our quantitative data indicate that the average number of cells counted per section in the control and the

2 Severinghaus, A. E., *Anat. Rec.*, 1934, **60**, 43.

3 Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 184.

4 Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1189.

† This extract, Follutein, was furnished by E. R. Squibb and Sons through the courtesy of Dr. J. J. Durrett.

‡ Progynon-B, furnished by the Schering Corp., was used.

TABLE I.

Frequency-Distribution Table Giving Percentage-Frequencies of the Various Cells and Their Means (M) in Percentage. The Mean Pituitary Weights of the Various Groups and the Mean Number of Cells and Mitoses Counted per Section in the Various Groups Are Given.

Level of Cells, %	Frequency—Distribution			Mean Level of Cell Types		
	Control	A.P.L.	Oestrin	Control	A.P.L.	Oestrin
Eosinophiles						
15 — 19.9	—	—	1			
20 — 24.9	—	—	—	M — 48.5	M — 47.1	M — 31.5
25 — 29.9	—	—	3	Cells counted per section—610	Cells counted per section—592	Cells counted per section—473
30 — 34.9	—	—	3			
35 — 39.9	—	—	1			
40 — 44.9	8	4	1			
45 — 49.9	7	6	1			
50 — 54.9	11	4	—			
55 — 59.9	4	—	—			
Basophile—Gran.						
0 — 1.9	1	14	10			
2 — 3.9	6	—	—	M — 5.4	M — 1.0	Practically none
4 — 5.9	11	—	—	Cells counted per section—68	Cells counted per section—12	
6 — 7.9	10	—	—			
8 — 9.9	2	—	—			
10 — 11.9	—	—	—			
Basophile—Non-gran.						
0 — 1.9	29	—	6			
2 — 3.9	1	5	3	M — 1.1	M — 4.5	M — 2.0
4 — 5.9	—	5	1	Cells counted per section—14	Cells counted per section—57	Cells counted per section—30
6 — 7.9	—	4	—			
8 — 9.9	—	—	—			
Chromophobe						
30 — 34.9	1	—	—			
35 — 39.9	5	—	—	M — 45.2	M — 47.4	M — 66.5
40 — 44.9	10	3	—	Cells counted per section—564	Cells counted per section—598	Cells counted per section—997
45 — 49.9	5	7	1			
50 — 54.9	9	4	1			
55 — 59.9	—	—	—			
60 — 64.9	—	—	1			
65 — 69.9	—	—	4			
70 — 74.9	—	—	2			
75 — 79.9	—	—	—			
80 — 84.9	—	—	1			
Mean Pituitary Weight	7.6 mg.	6.8 mg.	14.6 mg.			
Mitoses per section	5.0	5.0	46.9			
Mean number of total cells counted per section				1255	1259	1500

injected rats was practically the same (Table I). This would probably indicate that A. P. L. administration did not bring about an increase in the total number of cells in the gland.

Subcutaneous injections of oestrin for a period of 10 days induced a marked but variable weight increase in the pituitary; a finding previously reported by Hohlweg[5] and Halpern and

[5] Hohlweg, W., *Klin. Woch.*, 1934, **13**, 92.

[6] Halpern, S. R., and D'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 108.

D'Amour.[6] The mean was 14.6 mg., the range from 9 to 22 mg. The mean weight of the pituitaries of the controls was 7.6 mg. (Table I).

The anterior lobes of all rats receiving oestrin exhibited a marked loss of granules from the basophiles, furthermore the number of these cells counted per section was reduced. Certain less conspicuous changes were found in the eosinophiles and the chromophobes, the extent of which was associated with the degree to which the pituitary was increased in weight. Thus in 2 glands which weighed only 9 mg. the relative level of the eosinophiles was normal and they presented only slight granular loss. In the heavier glands many of the eosinophiles showed loss of granular material and their relative percentage was reduced. Eosinophiles showing loss of granules were often swollen and the remaining granules took a pale stain with orange G. The negative image of the Golgi apparatus was often enlarged. The above morphologic and quantitative changes became progressively more marked in the heavier pituitaries and in one gland which weighed 22 mg. the relative percentage of eosinophiles was reduced to 17.1%. Our quantitative data indicate that the number of eosinophiles counted in these enlarged pituitaries was reduced below normal but not to the degree to which the relative percentage was reduced (Table I). In the rats receiving oestrin the relative percentages and the number of the chromophobes counted were definitely increased (Table I). This increase was more or less proportional to the degree to which the pituitary was increased in weight and indirectly to the extent to which the granular cells were reduced in percentage. Many of the chromophobes were greatly enlarged and presented a fragmentary light blue cytoplasm. Others were smaller and had a denser blue cytoplasm. In both, the negative image of the Golgi apparatus was often hypertrophied.

In the rats receiving oestrin the number of mitoses found was greatly increased (Table I). Mitoses were most abundant in the chromophobes but were also found in the eosinophiles. In general mitoses were most abundant in those glands which were most increased in weight and presented the most marked histologic changes. The total number of cells counted per section was also increased (Table I).

These studies are of considerable interest in that they demonstrate that injection of oestrin induces a reaction in the anterior lobes of mature male rats similar to that induced by both oestrin and the A. P. L. factor in the normal mature female rat, *i. e.*, hypertrophy of the gland, marked loss of granules from the basophiles and less

significant loss of granules from the eosinophiles. On the other hand A. P. L. injection (the same amount as the females received) in the normal male rat induced loss of granules only from the basophiles.

8072 C

Effect of Feeding Desiccated Thymus upon Growth.

EATON M. MACKAY AND RICHARD H. BARNES.

From the Scripps Metabolic Clinic, La Jolla, California.

There are numerous studies in the literature concerning the effect of thymus gland feeding upon growth. Liddo[1] found that it had a harmful effect on all rats but was less harmful when fed with carbohydrates. As with many such studies the question of the completeness of the diets clouds the results and there is always the question of whether such effects are produced only by the thymus or may also result from feeding other animal tissues. This question has been examined.

Four rats were removed from each of 4 albino rat litters 30 days old. The 4 from one litter were females and the remainder males. One from each litter was placed upon each of 4 diets. The control diet was composed of cornstarch 41, commercial casein 20, dried brewer's yeast 10, Osborne and Mendel salt mixture 4, lard 15 and cod liver oil 10. One group was fed a mixture of 90% control diet plus 10% desiccated beef thymus (all of the desiccated material was prepared by The Wilson Laboratories, Chicago) and as additional controls groups were fed a mixture of 90% control diet plus 10% of desiccated beef liver and plus desiccated beef kidney, both very nuclear tissues.

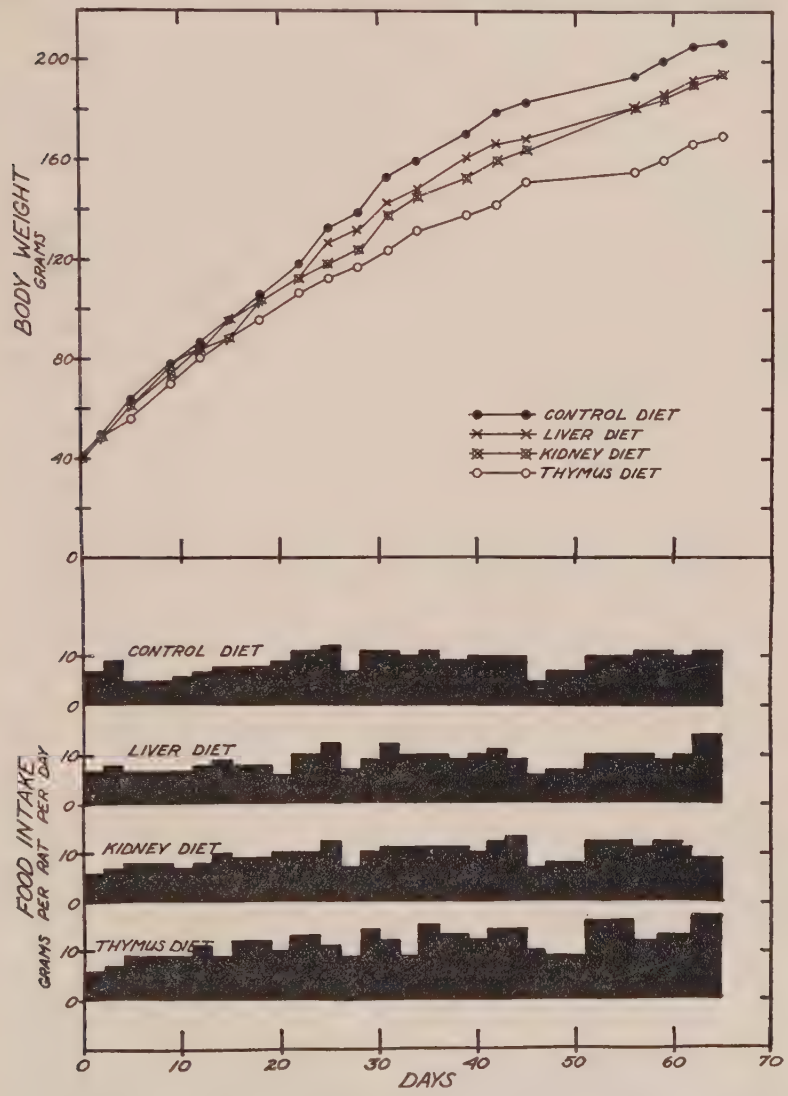
The relative influence of the addition to the diet of desiccated thymus, kidney and liver may be seen in Fig. 1 and we have confirmed this in other experiments. Both the kidney and liver material had essentially the same slightly deleterious influence on growth (see initial body weight and body length at death in Table I), while the desiccated thymus depressed the growth curve considerably more. The poor growth of the thymus-fed rats was in spite of a higher actual food intake than any of the other groups. The average food intake per rat per day for the control group was 9.0 gm., the liver

[1]Liddo, S., *Boll. Soc. ital. Biol. sper.*, 1933, **8**, 267.

TABLE I.

	Initial body wt. (gross)	Body wt. after 65 days (gross)	Body wt. after 48 hrs. starvation (gross)	Body surface on starved body wt.	Body length	Heart Weight			Adrenal Weight			Kidney Weight		
						Actual	Per 100 mm. body length	Per 100 sq.cm. body surface	Actual	Per 100 mm. body length	Per 100 sq cm. body surface	Actual	Per 100 mm. body length	Per 100 sq cm. body surface
	gm.	gm.	gm.	sq.cm.	mm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Control Diet	41	207	198	386	208	718	345	186	36	17	9	1406	677	364
Liver Diet	39	195	186	370	203	654	322	178	52	26	14	1330	656	360
Kidney Diet	40	195	179	360	203	888	437	247	65	32	18	1567	770	435
Thymus Diet	39	170	150	321	196	800	408	249	68	35	21	1487	760	469

fed group 9.6 gm., the kidney fed group 9.6 gm., and the thymus fed group 11.6 gm. We believe that the deleterious effect of these dried tissues on growth is due to their content of nuclear material.



We have found that the addition of yeast nucleic acid to their food impaired the growth of albino rats and the same thing has been reported as a result of feeding sodium nucleate.[2] If the figures of

2 Newburgh, L. H., and Johnston, M. W., *J. Clin. Invest.*, 1931, **10**, 156.

Schmid and Bessan[3] for purine nitrogen of fresh tissues are transferred to our desiccated products the kidney powder would contain 0.48%, the liver powder 0.47%, and the thymus powder 1.98% purine nitrogen. The relationship of these figures is the same as the influence of the respective dried tissues on the rate of growth.

The rats with kidney and those with thymus added to their diets had (Table I) abnormally heavy kidneys and hearts. Grossly, they were normal. All of the dried tissues when fed caused the adrenals to become heavier. This increased weight was apparently due to cortical enlargement.

Conclusions. The addition of a significant amount of desiccated beef liver or kidney to the diet of the albino rat impairs slightly the rate of growth. The deleterious influence of desiccated beef thymus is more pronounced. These effects are probably due to the nuclear material in these tissues for their influence is related to their content of purine nitrogen.

8073 C

Extracellular and Intracellular Water Loss during Suprarenal Insufficiency in the Dog.

GEORGE A. HARROP, WILLIAM M. NICHOLSON, LOUIS J. SOFFER, AND MARGARET STRAUSS.

From the Chemical Division of the Medical Clinic, Johns Hopkins University and Hospital, Baltimore.

In the cycle of cortical deficiency and recovery which may be induced in the suprarenalectomized dog by the withdrawal and subsequent readministration of suprarenal cortical hormone, two periods of diuresis may be discerned in balance experiments, in which the animal is offered an approximately constant food, fluid and sodium chloride intake. The first period of diuresis follows the withdrawal of the cortical extract and is associated with increased urinary excretion of sodium and of chloride. This important effect of suprarenal deficiency was first reported by Loeb and his coworkers, following suprarenalectomy in the dog.[1] The sodium

[3] Quoted by Brugsch, J., Gicht, in Kraus and Brugsch: *Specielle Pathologie und Therapie der innere Krankheiten*, Berlin, 1913, p. 228.

[1] Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., *J. Exp. Med.*, 1933, **57**, 775.

diuresis is associated with an excretion of extracellular water. During the period of insufficiency there may be no substantial alteration in the potassium, nitrogen, and phosphate balance, but the increased concentration of these substances in the blood plasma, coincidental with the fall in sodium and chloride, is usually well marked, and indicates a renal retention. To a certain extent, this increased concentration of phosphate and potassium in the plasma makes good the loss of osmotic pressure which would otherwise attend the fall in concentration of sodium and of chloride.

When the animal is revived from a condition of insufficiency by injections of cortical hormone, a second period of diuresis immediately takes place which is quantitatively much larger than the first. The body weight also continues to drop for a time after the cortical extract injections are resumed, even though the condition of the animal obviously is improving greatly and food is being taken eagerly. This second period of diuresis is associated with a marked increase in the urinary excretion of potassium, urea, and phosphates and with a diminished excretion of sodium.[2] Not only is the excretion of potassium augmented severalfold in amount as a result of the increased fluid excretion, but the urinary concentration of this ion is also raised. Coincidental with this urinary excretion of potassium, nitrogen, and phosphates, the concentration of plasma potassium, urea, and inorganic phosphate falls, and the concentration of plasma sodium and chloride rises to normal levels. The augmented urinary excretion of potassium and of nitrogen will account for the fall in concentration in the extracellular fluid, assuming the latter to approximate in volume of water 20% of the body weight, with subsequent contraction.

The potassium diuresis represents primarily an excretion of intracellular water, in part due to cell destruction and in part due to cell shrinkage, as shown by the quantities of potassium and of nitrogen excreted with respect to the associated water loss. The diuresis may reflect the effects of an alteration in cellular permeability taking place as a consequence of the contraction in volume of the extracellular water, with the resulting disturbance of normal cell metabolism. It must be regarded not as due directly to lack of the cortical hormone, but as a consequence of the disordered renal excretion of sodium with the simultaneous displacement of a large moiety of the extracellular water. It is noteworthy that the first consequence of the resumption of extract injections is an increased renal excretion

[2] Harrop, G. A., Soffer, L. J., Ellsworth, R., and Trescher, J. H., *J. Exp. Med.*, 1933, **58**, 17.

TABLE I.

Balance Experiment Showing Effect of Withdrawal and Subsequent Readministration of Suprarenal Cortical Hormone on a Bilaterally Suprarenalectomized Dog. Dog 132. Ground raw meat diet* with 300 cc. distilled water† and 1 gm. sodium chloride per day.†

Date 1935 Mar.	Weight kg.	Food Intake gm.	Extract cc.	Water‡ Balance cc.	Sodium Balance m.-eq.	Chloride Balance m.-eq.	Potassium Balance m.-eq.	Nitrogen Balance gm.	Urine Volume cc.
5-8	10.6	450	8	—	— 1.0	— 0.6	+ 1.6	— 0.1	455
				Extract injections stopped.					
8-9	10.5	450	0	—263	—66.0	—50.7	+18.3	+ 0.3	710
9-10	10.2	450	0	+ 52	— 5.3	+ 1.6	+12.6	+ 2.9	395
10-11	10.2	380	0	+ 35	— 3.7	—10.9	+ 7.2	+ 5.8	360
				Insufficiency. Extract resumed.					
11-12	10.1	0	50	—510	— 2.2	— 9.2	—56.4	—14.5	670
12-13	9.6	100	50	— 65	+16.0	+ 8.5	—15.9	— 5.1	250
13-14	9.7	150	15	+ 62	+16.1	+15.1	— 1.2	— 1.2	175
14-15	9.7	270	15	+182	+23.3	+18.3	+16.5	+ 2.2	235
15-16	9.7	390	10	+118	+20.5	+11.4	+11.8	+ 1.3	345
16-18	9.9	450	10	+ 22	—10.0	+ 6.2	+ 7.8	+ 0.1	435
18	10.1	450							

*The meat was ground, mixed for the entire period, a sample taken for chemical analysis, and the remainder weighed out into oiled paper parcels and preserved by refrigeration until used. The food was consumed within an hour after it was offered.

†Administered by stomach tube.

‡Represents the difference between intake (including water derived from food and extract) and output, considering the period March 5-8 as representing proper fluid balance.

The dog was catheterized daily. For details concerning conduct of metabolism experiments see Reference 2.

TABLE II.

Dog 132. Observations on the Blood and Heparinized Plasma (Arterial).

Date 1935 Mar.	Non-protein nitrogen mg. per 100 cc.	Urea mg. per 100 cc.	Sugar mg. per 100 cc.	Sodium m.-eq. per liter	Chloride m.-eq. per liter	Potassium m.-eq. per liter	Carbon dioxide content m.-eq. per liter	Proteins gm. %	Plasma (hematocrit) %	
5	36	20	73	144.8	111.1	4.3	30.1	6.0	62.2	
				Extract withdrawn March 8.						
11	180	140	95	133.0	101.5	10.6	17.4	6.9	46.5	T. 99, P. 34, stagger gait
				Extract resumed March 11.						
18	32	16	74	140.5	107.4	4.9	26.9	6.4	60.8	

of potassium and nitrogen, and that the retention of sodium and chlorides follows this primary effect, in most experiments.

Summary. The initial hemoconcentration which is observed in suprarenal insufficiency in the dog is due at least in part to a renal loss of extracellular fluid. It is followed during the early stage of repair by a further loss of water, primarily intracellular in origin, a reflection of cellular damage which is not made manifest until this

renal excretion takes place during the recovery phase. The first effect of the cortical hormone upon the animal in suprarenal insufficiency is to produce an increased urinary excretion of potassium, nitrogen, and phosphates, followed by retention of sodium and of chlorides.

The ultimate effect of the hormone is the restoration and maintenance of the proper plasma concentrations of potassium and sodium through regulation of their renal excretion. Hence, it plays a predominant rôle in the stabilization of a proper volume of extracellular fluid.

8074 C

Respiratory Exchange of Oxygen and Carbon Dioxide During Rebreathing from a Rubber Bag.

SIDNEY A. GLADSTONE* AND SIMON DACK. (Introduced by B. S. Oppenheimer.)

From the Medical Service of Dr. B. S. Oppenheimer, The Mount Sinai Hospital, New York City.

During rebreathing from a rubber bag, the nose being compressed with a spring clip so that the lungs and bag form a closed system, O_2 and CO_2 diffuse across the pulmonary epithelium, the direction and rate of diffusion depending on the relative tensions of these gases in the blood and alveolar air. This process has been studied in 18 experiments in 2 normal young men. After the subject had sat in a chair for 10 minutes, the metabolic rate was determined. The subject then expired through a side tube to residual air (an alveolar sample being taken) and then rebreathed from the rubber bag 9 times in 22.2 seconds, being guided by spoken directions from an accurately timed phonograph record. Each inspiration emptied the bag and each expiration was as deep as possible. During the rebreathing alveolar samples were drawn into evacuated tubes at the end of breath No. 3, 6, and 9, dividing each experiment into 3 intervals of 8.0, 7.0, and 7.2 seconds in the order named.

The results of the experiments are presented in tabular form. The contents of the rebreathing bag are noted. The initial volume of the lung-bag system is obtained by adding the gas volume of the bag and the residual lung volume of the subject. In most experi-

*Richard and Ella Hunt Sutro Fellow for Cardiovascular Research.

TABLE I.

Subject		O_2 cons. cc./min.	Barometer	Temperature		Volume	Change in volume		Period	O_2 absorbed			CO_2 discharged			R.Q. I	R.Q. II	R.Q. III
	cc.																	
D	air 2400				1	3994	+24	+22	1	89			111			1.25		
					2	3982	—12	—12	2		52			40			.77	
					3	3954	—28	—29	3			38			11			.29
D	air 2400				1	3991	+21	+20	1	93			113			1.22		
					2	3970	—21	—22	2		65			43			.61	
					3	3941	—29	—28	3			37			9			.24
D	air 2280 O_2 120	296	770	20	1	3974	+ 4	+ 1	1	95			96			1.01		
					2	3963	—11	—10	2		49			39			.80	
					3	3938	—25	—24	3			36			12			.33
D	air 2280 O_2 120	300	768	26	1	3976	+ 6	+11	1	92			103			1.12		
					2	3965	—11	—11	2		53			42			.79	
					3	3937	—28	—29	3			38			9			.24
D	air 2280 CO_2 120	269	778	25.5	1	3924	—36	—40	1	70			30			.43		
					2	3885	—39	—41	2		55			14			.25	
					3	3852	—33	—32	3			50			18			.36
D	air 2280 CO_2 120	278	775	24	1	3936	—34	—34	1	62			28			.45		
					2	3903	—33	—33	2		44			11			.25	
					3	3878	—25	—28	3			45			17			.38
D	air 2280 CO_2 120	234	775	24	1	3937	—33	—33	1	56			25			.45		
					2	3912	—25	—27	2		47			20			.43	
					3	3879	—33	—31	3			16			—17			
D	air 2280 CO_2 120	262	767	27	1	3896	—74	—45	1	71			26			.37		
					2	3863	—33	—32	2		56			24			.43	
					3	3832	—31	—30	3			18			—12			
D	air 2280 CO_2 120	246	766	27	1	3926	—44	—43	1	67			24			.36		
					2	3888	—38	—28	2		48			20			.42	
					3	3861	—27	—25	3			12			—13			
D	air 2150 CO_2 250	294	763	20	1	3882	—88	—95	1	59			—36					
					2	3825	—57	—55	2		45			—10				
					3	3786	—39	—41	3			38			—3			

TABLE I—Continued.

Subject		O_2 cons. cc./min.	Barometer	Temperature		Volume	Change in volume		Period	O_2 absorbed		CO_2 discharged		R.Q. I	R.Q. II	R.Q. III
	cc.															
D	air 2150	256	763	21	1	3879	—141	—144	1	73		—71				
	CO_2 300				2	3811	—68	—66	2		45		—21			
					3	3764	—47	—48	3		42		—6			
G	air 2400	301	775	23	1	4015	+45	+43	1	61		104		1.71		
					2	3987	—28	—27	2		65		38		.59	
					3	3931	—56	—37	3		82		45			.55
G	air 2400				1	4013	+43	+33	1	74		107		1.45		
					2	4000	—13	—14	2		45		31		.69	
					3	3936	—37	—35	3		60		25			.42
G	air 2400				1	4015	+45	+44	1	58		102		1.76		
					2	4003	—12	—11	2		54		43		.80	
					3	3966	—37	—38	3		64		26			.41
G	air 2280	279	778	19	1	3936	—34	—32	1	43		11		.26		
	CO_2 120				2	3893	—43	—40	2		56		16		.29	
					3	3834	—59	—60	3		80		20			.25
G	air 2280	299	778	185	1	3937	—33	—33	1	47		14		.30		
	CO_2 120				2	3897	—40	—39	2		55		16		.29	
					3	3868	—29	—40	3		60		20			.33
G	air 2150	267	775	21	1	3868	—102	—105	1	53		—52				
	CO_2 250				2	3795	—73	—72	2		50		—22			
					3	3749	—46	—48	3		59		11			.19
G	air 2150	270	775	22	1	3868	—102	—101	1	57		—44				
	CO_2 250				2	3790	—78	—78	2		56		—21			
					3	3734	—56	—57	3		66		9			.14

ments the initial volume was 2400 + 1570 = 3970 cc. The volume changes in the system were calculated from the changes in percentage of nitrogen which is assumed to take a negligible part in the transepithelial gaseous exchange. The volume changes may also be calculated from the difference of O_2 absorbed and CO_2 discharged. The 2 sets of findings listed side by side are in close agreement with a few exceptions probably due to errors in technique or gas analysis. From the total volume of the system and the percentage of each gas, the amount of each gas at the time of sampling may be calculated, and the change in volume of O_2 and CO_2 for each period. From the latter the R. Q. is calculated.

The findings recorded may be understood if one bears in mind first that the alveolar CO_2 is reduced to less than half its normal value when the residual air is diluted by the first inspiration of 2,400 cc. of air from the bag. This lowering of the alveolar CO_2 quickens the diffusion of CO_2 from the blood to the lungs, but as the alveolar CO_2 rises the rate of diffusion is depressed. This fact has been demonstrated by the previous addition of CO_2 to the bag. Furthermore, the demonstration subsequently made that appreciable amounts of blood recirculate within 10 to 12 seconds (see next paper) after rebreathing has begun, indicates that we are dealing with unaltered "mixed venous blood" only during the first period of these experiments, and that the gaseous tensions of the blood reaching the lungs in the second and third periods will be influenced not only by the deep rapid breathing but by the gaseous content of the bag to which the recirculating blood has been previously exposed. Hence the marked change in O_2 absorption observed in successive intervals although the latter differ only slightly in duration.

From comparison of the amounts of oxygen absorbed in the same subject during the first period it may be seen that the addition of O_2 to the bag contents has little effect on the amount of oxygen absorbed, but the latter is depressed if CO_2 is added. Both of these observations are in keeping with the oxyhemoglobin dissociation curves of human blood.[1]

The rate of oxygen absorption during the first period (8 seconds, *i. e.*, before recirculation begins) is about twice the rate observed during the quiet breathing of the metabolism test which preceded the rebreathing experiment. This is due to the increased blood flow through the lungs produced by the deep rapid breathing.

[1] Bohr, C., Hasselbach, K. A., and Krogh, A., *Skand. Arch. f. Physiol.*, 1904, **16**, 402.

As the rebreathing of air continues, the alveolar CO_2 rises, the discharge of CO_2 decreases; the R. Q. exceeds unity in the first period, but quickly falls in the second and third periods. The total volume of the lung-bag system increases during the first period, decreases thereafter. These volume changes explain the difficulties experienced by certain workers in demonstrating the uniform percentage concentration of an inert gas in a lung-bag system,[2, 3] and also the inadequacy of any mixing criteria which are based on percentage concentration of the inert gas without due consideration of the concomitant changes in total volume.

8075 P

The Factor of Recirculation in Acetylene Method for Determination of Cardiac Output.

SIDNEY A. GLADSTONE.* (Introduced by B. S. Oppenheimer.)

From the Medical Service of Dr. B. S. Oppenheimer, The Mount Sinai Hospital, New York City.

Hamilton, Spradlin, and Saam[1] on the basis of animal experiments have concluded that the results of the acetylene method[2] for determining the cardiac output are vitiated by the return of acetylene to the lungs before the procedure is completed. After using the Marshall-Grollman method for one year,[3] the present writer felt the necessity of studying this question in human subjects. If and when recirculation of acetylene-laden blood occurs in appreciable amounts, it will become manifest by a reduction in the rate of diffusion from the lungs into the blood, a reduction in the diffusion constant K of the system, and a rise in the calculated arterio-venous oxygen difference. In 11 rebreathing experiments on 2 subjects, these data were obtained for successive short intervals during each experiment by drawing several alveolar samples, the breathing and sampling being directed by an accurately timed phonograph record.

2 Lundsgaard, C., and Schierbeck, K., *Am. J. Physiol.*, 1923, **64**, 210.

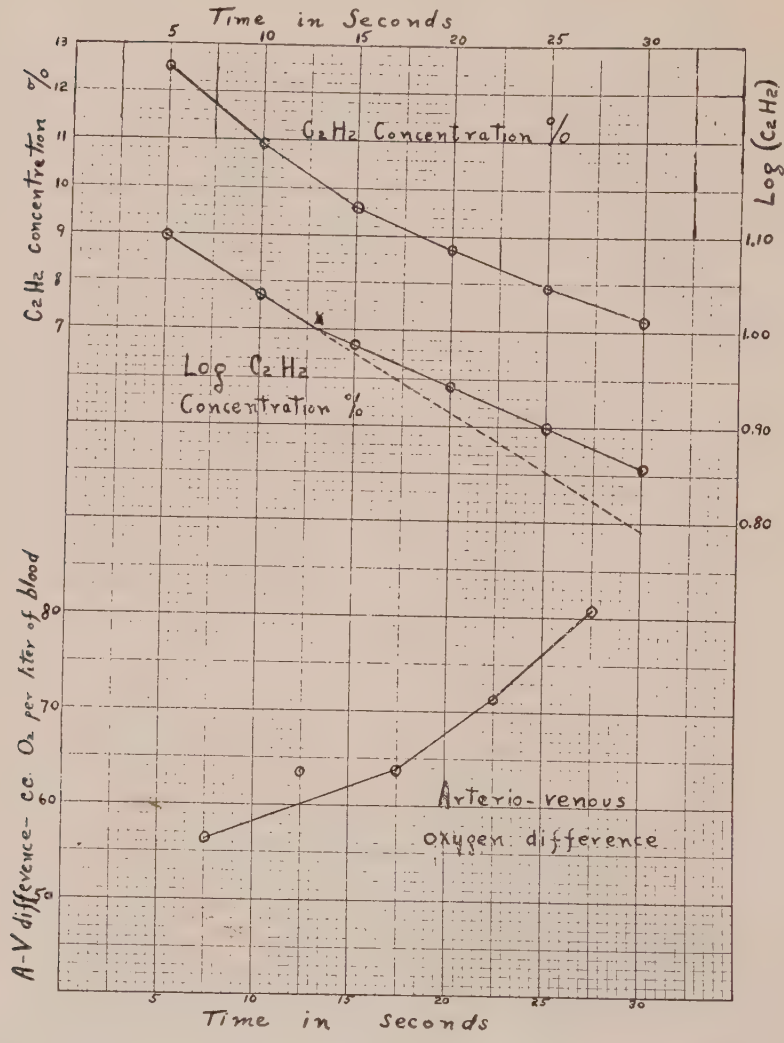
3 Grollman, A., and Marshall, E. K., *Am. J. Physiol.*, 1928, **86**, 110.

* Richard and Ella Hunt Sutro Fellow for Cardiovascular Research.

1 Hamilton, Spradlin, and Saam, *Am. J. Physiol.*, 1932, **100**, 589.

2 Grollman, A., *The Cardiac Output of Man in Health and Disease*, Baltimore, 1932.

3 Gladstone, S. A., *Arch. Int. Med.*, 1935, **55**, 533.



The results of one typical experiment are graphically presented. If the blood flow through the lungs is fairly constant during the rebreathing experiments, the rate of diffusion of the acetylene from lungs to blood should be proportional to the constantly decreasing alveolar concentration of the gas. Charted as the ordinate against time as the abscissa, the concentration of C_2H_2 should follow an exponential or logarithmic curve, and the logarithms of the concentration should follow a straight line, the slope of which will indicate the rate of diffusion of the gas. In the experiments performed, the break in this line with flattening of the curve indicating a depression in the rate of diffusion of the gas due to recirculation in appreciable

amounts, has occurred at 10 seconds or promptly thereafter. This rapid recirculation is undoubtedly due to the increase in blood flow through the lungs occasioned by the deep rapid breathing of the procedure. The increased blood flow is also indicated by the volumes of O_2[4] and C_2H_2 removed from the lungs during the first 10 seconds before recirculation begins. There can be no doubt that recirculation occurs in the Marshall-Grollman procedure not only before the test is over (23 seconds) but even before the first sample is drawn (at 15 seconds). The ability to obtain good checks with the Marshall-Grollman procedure (an ability which has differed widely in the hands of different workers) will depend on timing the samples in exactly the same way in repeated experiments so that the errors due to recirculation (which are partly compensated by the error due to abnormal quickening of the blood flow beyond the metabolic needs of the tissues with consequent increase in the O_2 content of the mixed venous blood) will be fairly constant. The writer has been gradually and unwillingly forced to the conclusion that the results of the Marshall-Grollman method are heavily weighted with errors which compensate each other to a varying degree under different conditions, rendering the absolute figures obtained sometimes correct but never completely reliable, and rendering the method insensitive to the detection of small differences, because the size of the errors involved may vary even more than the function to be measured.

8076 P

A Modified Foreign-Gas Method for Determination of Cardiac Output in Man.

SIDNEY A. GLADSTONE.* (Introduced by B. S. Oppenheimer.)

From the Medical Service of Dr. B. S. Oppenheimer, The Mount Sinai Hospital, New York City.

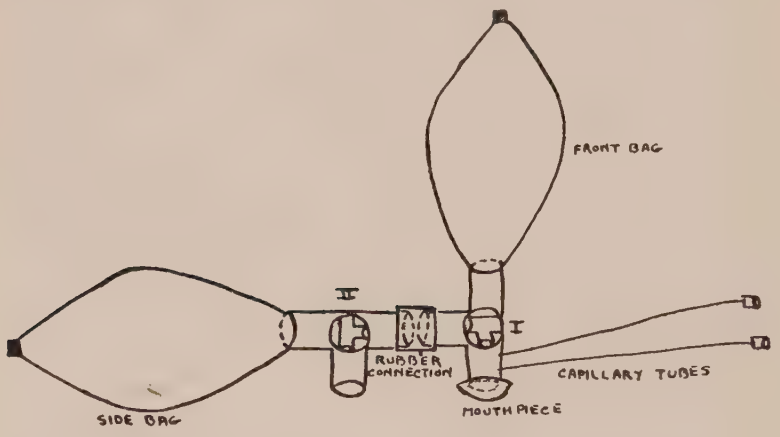
The application of the foreign-gas principle for the determination of the arteriovenous oxygen difference and cardiac output in man is beset by two difficulties; first, the attainment of a homogeneous mixture in the gaseous system with which the arterial blood is assumed to be in equilibrium; second, the termination of the pro-

4 Preceding publication.

* Richard and Ella Hunt Sutro Fellow for Cardiovascular Research.

cedure before recirculation of blood takes place. The demonstration that the latter occurs within 10 seconds[1] renders unavailable the mixing technique of Marshall and Grollman[2] which requires 15 seconds, and *a fortiori* their rebreathing procedure which lasts for 23 seconds.

To restrict the procedure to the time available, the writer has introduced a method for attaining homogeneity of composition that can be finished in 3½ to 4 seconds, and is accomplished by dividing the inhaled foreign-gas mixture into 2 portions, such that the gas filling the dead space approximates in composition the alveolar contents, and further by washing out and discarding the contents of the dead space before the first alveolar sample is drawn.



The apparatus consists of 2 three-way aluminum valves (bore 2 cm.), 2 rubber breathing bags (capacity 4 liters) connected as shown in diagram. Lead tubes 15 cm. in length and of capillary (0.5 mm.) bore permit the drawing of samples from the mouth side of the valve into evacuated sampling tubes. Bag contents are

Front Bag		Side Bag
825 cc.	air	1300
55	CO_2	50
120	C_2H_2	500
	O_2	150
Total 1000		Total 2000

After the nose clip is applied, the subject expires to residual air, and then inspires the contents of the side bag followed by the contents of the front bag. The experimenter turns valve I so that

[1] Preceding publication.

[2] Marshall, E. K., and Grollman, A., *Am. J. Physiol.*, 1928, **86**, 117.

approximately the first liter of the ensuing expiration is discarded into the side bag, the rest of the expiration being directed into the empty front bag and at the end of this deep expiration the first sample is drawn. This preliminary period can be finished in 3½ to 4 seconds. Two more deep breaths requiring about 5 seconds are taken and the second sample is drawn at the end of the last breath, 8½ to 9 seconds after the beginning of the test.

The adequacy of the mixing procedure may be tested by drawing the first sample simultaneously at opposite ends of the rebreathing (front) bag and pairing each sample with the final sample for 2 separate calculations of the A-V difference. In 7 experiments (Table I) this double sampling has been done. The average dif-

TABLE I.
Effect on Different Individuals of Drawing First Alveolar Sample from Opposite Ends of Rebreathing Bag.

Pulse Rate per Min.	Blood Pressure Mm. of Hg.	O Consumption, cc. per Min.	Arteriovenous O Difference, cc. per L. of blood A	B	Difference between A and B	Cardiac Output Liters per Min. I	II
64	108/65	256	49.7	53.9	4.2	5.2	4.8
76	106/75	284	50.4	49.7	0.7	5.6	5.7
68	112/72	258	51.3	52.3	1.0	5.0	4.9
76	115/70	259	43.8	50.2	6.4	5.9	5.2
70	105/72	247	43.6	46.0	2.4	5.7	5.4
88	116/80	252	43.1	45.8	2.7	5.9	5.5
88	120/76	270	50.3	50.1	0.2	5.4	5.4
				Average	2.5	5.5	5.3

ference of about 5% may be attributed to errors of mixing and gas analysis. Since the latter alone may account for the variations observed, the error due to imperfect mixing is either negligible or absent.

In the gas analysis, to reduce the error due to absorption of C_2H_2 during removal of CO_2[3] the author has introduced for absorption of CO_2 a solution containing 40 gm. of NaOH and 6 gm. of NaCl per 100 cc. of solution.

The present technique is an application of the foreign-gas method, identical in principle and method of calculation with the nitrous oxide method of Krogh and Lindhard[4] but following more closely the rebreathing procedure of Marshall and Grollman. The essential features of the method described are the shortening of the technique to 8½ to 9 seconds, and the rapid mixing procedure (3½ to 4 seconds) which has made that shortening possible.

3 Gladstone, S. A., *Arch. Int. Med.*, 1935, **55**, 533.

4 Krogh, A., and Lindhard, J., *Skand. Arch. für Physiol.*, 1912, **27**, 100.

8077 P

Study of Cholesterol Fractions in Acute Infections.

A. V. STOESSER. (Introduced by Irvine McQuarrie.)

From the Department of Pediatrics, University of Minnesota.

In previous communications[1, 2] the plasma lipids were reported low during the height of acute infections. Cholesterol values were found to fall to definitely subnormal levels during the febrile period of the illness, returning up to or within the normal range during convalescence. This change in the cholesterol content of the blood serum has repeatedly been considered as a possible significant factor in the defense mechanism of the host against the invading organisms of an acute infection. Cholesterol is known to combine readily with bacterial toxins thereby rendering them less toxic.

Since cholesterol, however, exists in the blood as free cholesterol and in combination with fatty acids as ester cholesterol, it is of special interest to determine the individual changes which occur in the levels of the various plasma cholesterol fractions in acute infections. Twelve children ranging in age from 18 months to 13 years were chosen for this study. Six of the subjects had rather extensive infections of the respiratory tract, and the remainder were ill with pneumonia. The first blood sample was obtained at the height of

TABLE I.
The Plasma Cholesterol Fractions in Infections of the Respiratory Tract.

Case No.	Total cholesterol	Cholesterol esters	Free cholesterol
	Mg. per 100 cc. serum		
	The height of the disease		
1	142	50	92
2	138	47	91
3	130	66	64
7	112	47	65
8	94	32	62
12	160	71	89
Aver.	129	52	77
	The period of convalescence		
1	224	155	69
2	226	125	101
3	218	138	70
7	201	111	90
8	234	154	80
12	220	180	40
Aver.	220	143	75

[1] McQuarrie, Irvine, and Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1281.

[2] Stoesser, A. V., and McQuarrie, Irvine, *Am. J. Dis. Child.*, 1935, **49**, 658.

TABLE II.
The Plasma Cholesterol Fractions in Pneumonia.

Case No.	Total cholesterol	Cholesterol esters	Free cholesterol
	Mg. per 100 cc. serum		
	The height of the disease		
4	160	71	89
5	126	71	55
6	122	55	67
9	110	43	67
10	101	55	46
11	158	74	84
Aver.	129	61	68
	The period of convalescence		
4	280	167	113
5	235	181	54
6	232	167	65
9	226	137	89
10	214	167	47
11	236	154	82
Aver.	237	162	75

the disease, the average time being the sixth day of the illness. The second sample of blood was drawn during the convalescent period on or about the ninth day of normal temperature. All blood samples were collected between 12 and 16 hours after a meal. Bloor's methods[3, 4, 5] were used to determine the total, ester, and free cholesterol values.

The results are summarized in Tables I and II.

The total cholesterol values are much less during the height of the disease than in the period of convalescence. This reduction in acute infections is due to a marked fall in the ester cholesterol, while the free cholesterol content of the blood serum undergoes very little change. The underlying cause of this definite change in the ester fraction is the subject of further investigations which are in progress.

[3] Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

[4] Bloor, W. R., and Knudson, Arthur, *J. Biol. Chem.*, 1916, **27**, 107.

[5] Bloor, W. R., personal communication to the author.

8078 P

Effect of Acute Infection on Iodine Number of Serum Fatty Acids.

A. V. STOESSER. (Introduced by Irvine McQuarrie.)

From the Department of Pediatrics, University of Minnesota.

The marked fall in the ester cholesterol content of the serum during the height of acute infections observed in the preceding paper[1] is paralleled by a moderate drop in the plasma total fatty acids. Little significance has been attached to the changes which occur in the fatty acids of the blood in infection. However, recent interest in the unsaturated fatty acids in relationship to nutritional disorders has given stimulus to studies concerning the possible influence of acute infection on the unsaturation of the serum fatty acids. Investigations have been started.[2, 3]

This paper deals with a study including 12 children with acute infections. They are the same subjects which were carefully followed in the cholesterol study. All blood samples drawn during and after the febrile period were collected between 12 and 16 hours after a meal. The first blood sample was obtained on the sixth day of the disease, and the second blood sample on the ninth day of

TABLE I.
Iodine Numbers of Serum Fatty Acids in Infections of the Respiratory Tract.

Case No.	Total fatty acids Mg. per 100 cc. serum	Iodine absorbed Mg. per 100 cc. serum	Iodine number
	The height of the disease		
1	334	342	102
2	217	223	103
3	352	303	86
7	346	327	94
8	180	152	85
12	432	476	110
Aver.	310	304	96
	The period of convalescence		
1	437	524	120
2	332	475	143
3	411	398	97
7	368	420	114
8	274	322	117
12	494	592	119
Aver.	386	455	118

[1] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1324.

[2] Achard, C., Grigaut, A., Leblanc, A., and David, Marcel, *J. de physiol. et de path. gen.*, 1928, **26**, 415.

[3] Stoesser, A. V., and McQuarrie, Irvine, *Am. J. Dis. Child.*, 1935, **49**, 658.

TABLE II.
Iodine Numbers of Serum Fatty Acids in Pneumonia

Case No.	Total fatty acids Mg. per 100 cc. serum	Iodine absorbed Mg. per 100 cc. serum	Iodine number
	The height of the disease		
4	333	326	98
5	298	294	99
6	214	192	95
9	329	269	81
10	286	337	118
11	387	337	87
Aver.	307	292	96
	The period of convalescence		
4	420	520	123
5	394	494	125
6	272	319	117
9	367	363	98
10	364	426	117
11	432	447	103
Aver.	375	428	114

convalescence. Bloor's methods[4] were used to determine the blood lipids. The Rosenmund-Kuhnhenn method[5] as modified by Page, Pasternak and Burt[6] was used to determine the iodine absorption of the serum fatty acids.

The results from the study are shown in Tables I and II.

The iodine absorption values are definitely lower during the height of the disease. This change accompanied by a moderate fall in the total fatty acids yields an average iodine number of the serum fatty acids which is significantly lower during the febrile period of the illness than during the afebrile period of convalescence. This indicates that the serum fatty acids are less unsaturated in acute infection, thereby constituting a rather important factor to be considered in all investigations dealing with changes in the serum fatty acids. In addition, the shift in the ratio of unsaturated fatty acids to saturated fatty acids may be significant in throwing more light on the chemical studies involving the immune processes which occur in acute infections.

[4] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[5] Rosenmund, K. W., and Kuhnhenn, W., *Z. f. unter. d. Nahr. d. Nahr. u. Genuss.*, 1923, **46**, 154.

[6] Page, H. H., Pasternak, L., and Burt, M. L., *Biochem. Z.*, 1930, **223**, 445.

8079 P

Angiography by Use of Viscous Radiopaque Solutions.

MAURICE BERCK. (Introduced by B. S. Oppenheimer.)

From the Laboratories and X-ray Department, Mount Sinai Hospital, New York City.

The delineation of the vascular system of the cerebrum and of the extremities has been accomplished with the use of thorium solution. This substance has the disadvantages of non-excretion and subsequent storage in the reticulo-endothelial system with dangerous sequelae of radio-activity. Sodium iodide in solutions of high concentration has likewise been used for these purposes, but has the disadvantage of the reaction of iodism.

Several authors[1-4] have outlined the vascular system of the lungs using the technique of intra-auricular catheterization and the subsequent injection of radiopaque solutions of concentrated sodium iodide and uroselectan directly into the interior of the right auricle. This procedure is formidable and hazardous, but necessary to these investigators because of the extremely rapid dispersal and dilution of their radiopaque solutions when injected into the peripheral venous circulation.

It occurred to the author that a high increase in viscosity of a radiopaque solution would enable one to avoid the necessity of intra-auricular catheterization because the rate and degree of dispersion and dilution of a viscous solution injected into an arm vein would be appreciably lessened and angiography could thus be made practicable.

Accordingly the innocuous, commonly used, 50% glucose solution was chosen as the viscous base and hippuran as the radiopaque substance. It was found that a 100% solution of the hippuran in 50% glucose solution could be effected. Injections of this viscous mixture were made into the arm vein of 4 rabbits and 2 dogs.

By fluoroscopy in 4 rabbits and 2 dogs the viscous radiopaque column of fluid could be visualized entering the heart, pulmonary arteries, outlining the thoracic aorta, and, in the rabbits, yielding excellently depicted angiographs (arterial) of the liver, spleen and kidneys. Two cc. of the 100% solution per kilo body weight was the amount injected. The animals survived without reaction.

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1 Moniz and Carvalho, *Bull. Acad. Med.*, 1931, **105**.

2 Ravina, Sonnie, Benzaquen, *Presse Medicale*, 1932, **15**, 287.

3 Conte and Costa, *Radiology*, 1933, **21**, 461.

4 Carvalho and Moniz, *Acta Radiol.*, 1933, **14**, 433.

Further studies are now being made on toxicity and the dosage suitable for human injection with the hope that an innocuous mixture can be effected combining high viscosity and radiopacity which is suitable for safe and practical angiography.

8080 P

Relation of the Phospholipins to the Reactivity of Antipneumococcus Sera.

FRANK L. HORSFALL, JR., AND K. GOODNER.

From the Hospital of the Rockefeller Institute for Medical Research, New York.

Hardy and Gardiner's method[1] for the removal of lipoids from sera is based upon the fact that extraction of protein solutions by alcohol or alcohol-ether at low temperatures does not cause denaturation. Using this method, Hartley[2] has shown that the removal of lipoids from certain antisera apparently abolishes their *in vitro* reactivity. Felton[3] has extracted antipneumococcus horse serum in a similar manner, and has demonstrated that the removal of lipoids does not diminish protective action.

These findings have been confirmed for Type I antipneumococcus horse serum. Extracted sera fail to agglutinate homologous type pneumococci and to give a precipitate with the specific capsular polysaccharide. *In vivo,* however, they show the presence of protective antibody in unaltered concentration.

Lipoid extraction was carried out in the following manner: The antiserum was introduced, with stirring, into 10 volumes of absolute alcohol at —10°C. After 6 hours' extraction the precipitate was collected by centrifugation at a temperature below —2°C., and again extracted with an amount of chilled absolute alcohol equal to that first used. After 12 hours the precipitate was collected in the same manner, and was then extracted with anhydrous ether for 10 hours at —10°C. and for a second time with anhydrous ether for 10 hours at room temperature. The precipitate was then collected and freed of ether by vacuum distillation, and finally was dissolved in an amount of saline equal to the original serum volume. The resulting solution does not differ in appearance from untreated serum.

1 Hardy, W. B., and Gardiner, S., *J. Physiol.,* 1910, **40**, lxviii.

2 Hartley, P., *Brit. J. Exp. Path.,* 1925, **6**, 180.

3 Felton, L. D., and Kauffmann, G., *J. Immunol.,* 1933, **24**, 543.

Although failing entirely *in vitro,* the extracted antipneumococcus horse serum brought about agglutination *in vivo* as well as unextracted serum. As a consequence of this observation extracted immune horse serum was injected intraperitoneally into mice and the peritoneal fluid was withdrawn after 30 minutes. This recovered fluid possessed the capacity to agglutinate homologous type pneumococci. This result suggested that the modified antibody had in some manner been reactivated by a substance in the peritoneal fluid. In an attempt to duplicate this effect *in vitro* a series of separate purified lipoids was employed, and it was found that the addition of minute amounts of lecithin at pH 6.0 completely restored *in vitro* activity.

Antipneumococcus rabbit serum, on the other hand, retains all of its *in vitro* reactive properties after extraction by alcohol-ether in the cold. If, however, the process is altered to include 2 extractions with petroleum ether at room temperature after treatment with alcohol, the *in vitro* reactivity of immune rabbit serum is almost entirely abolished. Its protective action, however, remains unchanged. It was found that in this instance the *in vitro* reactivity of the extracted rabbit serum could be restored only by the addition of cephalin.

It is of some interest that although these sera can be specifically reactivated by certain lipoids they are not reactivated by the lipoid material originally extracted from them. In this connection it has been found that the addition of minute quantities of free cholesterol to the extracted sera, prior to the addition of either cephalin or lecithin, prevents the reactivation in both instances.

It has not yet been possible to determine whether or not these latter lipoids are essential constituents of the antibody molecule, although 2 facts may point in this direction. The first is the specific character of the reactivating process. The second is the change in the solubility of the immune fraction which is present in extracted horse serum, for this is no longer precipitated by ten times dilution with distilled water.

Antipneumococcus sera from several animal species have been studied from this point of view. Those from the guinea pig and the rat apparently belong to the rabbit or "cephalin" group, while those from the goat, the mouse, and man belong to the horse or "lecithin" group.

The results presented in this summary are a further demonstration of the wide differences in the properties of antipneumococcus sera derived from the rabbit and the horse.

8081 P

A Test for Diagnosis of Thrombo-Angiitis Obliterans.*

CHARLES GOODMAN AND MAURICE BRODIE.

From the Department of Bacteriology, New York University Medical School.

In a previous report, one of us (Goodman) had outlined the evidence for the belief based upon epidemiological studies of others that thrombo-angiitis obliterans was a late manifestation of typhus fever. Since that time, Zinsser has pointed out in studies of Brill's disease that typhus infection may remain latent in the body for an indefinite length of time.

In a previous communication we have pointed out that a formalized Rickettsia suspension seemed to give a positive skin reaction in individuals with a previous typhus infection. Therefore we attempted this skin test in a number of cases of thrombo-angiitis obliterans in order to determine whether or not they had had a previous infection of typhus fever.

Accordingly, 14 males clinically diagnosed as thrombo-angiitis obliterans, some with and some without gangrene, were given intracutaneously 0.1 to 0.2 cc. of formalized Rickettsia suspension as used previously. At the same time for control, an injection of a similar amount of Proteus X19 filtrate was also likewise injected at a different site. A group of 12 controls included 2 with arterio-

TABLE I.

Type of Case	Age	Reaction to Rickettsia Suspension	Reaction to Proteus X19 Filtrate	Interval between Infection and Test	Size of Reaction cm.
T.A.O.	47	+	—	8 yr.	1
T.A.O.	30	++	—		4
T.A.O.	21	+			1½
T.A.O.	52	++			4
T.A.O.	44	++	—		4½
T.A.O. (gangrene)	47	+	Pro. Urt.		1½
T.A.O.	52	++	—		2
T.A.O.	33	+			1
T.A.O.	35	+	—		1
T.A.O.	57	+	—		1
T.A.O.	36	+	—		¾
T.A.O.	48	+	—		1½
T.A.O.	47	+	—		1
T.A.O.	53	+	—	6 mo.	1

T.A.O.: Thrombo-angiitis obliterans.
Cases furnished through courtesy of Dr. S. Samuels, Stuyvesant Clinic.

* Aided by grants from Messrs. N. Jonas and J. H. Post and Dr. F. L. Babbott, Jr.

sclerosis. The site of the injections was examined as in the previous study after 24 to 48 hours following the injection. The results are enumerated in Table I, where a negative reaction indicates no erythema or induration, and a + reaction a wheal or raised, reddened, indurated area ½ cm. to 1 cm. in diameter, and a ++ reaction from 1 cm. to 3 cm.

The cases diagnosed clinically as thrombo-angiitis obliterans show a positive skin test with Rickettsia organisms which suggests the possibility of previous typhus fever infection. Moreover, a number of the cases diagnosed as thrombo-angiitis obliterans came from typhus infected areas.

Of course the series is small and likewise further controls are required by skin-testing other vascular conditions as well as skin tests with further control suspension, such as tunica vaginalis and guinea pig testicle. Such experiments are now under way.

We have submitted evidence based upon a skin test with formalized Rickettsia suspension for the possibility that thrombo-angiitis obliterans is related to a previous typhus fever infection.

8082 P

A Skin Test Indicating a Previous Typhus Infection.

CHARLES GOODMAN AND MAURICE BRODIE.

From the Department of Bacteriology, New York University Medical School.

The recent studies of Zinsser[1] concerning the possible relationship of Brill's disease to a previous typhus fever infection suggests the importance of determining by a simple test a previous infection with typhus fever.

Inasmuch as many such patients are of foreign birth, and in whom infection had probably occurred some years previous, the clinical history is not always readily obtained. Likewise, a Weil-Felix reaction usually becomes negative with, or soon after convalescence and therefore cannot be used for this purpose.

The present work described the use of formalized Rickettsia suspension for this purpose. Fleck and Kurkowski[2] found that filtrates of Proteus X 19 were ineffective. Our tests were carried out with

[1] Zinsser, H., *Am. J. Hyg.*, 1934, **20**, 513.

[2] Fleck and Kurkowski, *Z. fur Immunitatsforsch.*, 1931, **72**, 282.

a formalized Rickettsia suspension made from tunica vaginalis scrapings kindly furnished us by Zinsser of Harvard University.

Accordingly, a series of 12 males who had had typhus fever, the diagnosis having been established 6 months previously in one case, and in the remainder from one year to 8 years were given the test about to be described. These cases were obtained through the courtesy of Dr. J. Rosenbluth, of the Bureau of Communicable Diseases of the New York City Department of Health. In all these cases the diagnosis had been confirmed by a positive Weil-Felix reaction with the exception of one in which the Weil-Felix had not been carried out.

For the control, 10 adults with no previous history of typhus fever infection were given a similar skin test. The test consisted of an intracutaneous injection of 0.1 to 0.2 cc. of formalized Rickettsia suspension. The patients were observed 24 to 48 hours after the injection. None of the controls gave any reaction, whereas all the 12 with the previous history of typhus infection showed an erythematous indurated area from 1 cm. to 4 cm. in diameter. None of the controls showed any skin reaction whatsoever. Another individual similarly tested one week after convalescence, likewise gave a positive reaction 2 cm. in diameter.

TABLE I.

Type of Case	Age	Reaction to Rickettsia Suspension	Reaction to Proteus X19 Filtrate
Normal	45*	—	—
	40*	—	—
,,	38	—	—
,,	29	—	—
,,	33	—	—
,,	28	—	—
,,	31	—	—
,,	30	—	—
,,	30	—	—
,,	32	—	—
,,	39	—	—
Arterio-sclerosis	64	—	—
,,	58	—	—

*Females.

These results suggest that patients recovered from typhus fever display a sensitivity to Rickettsia bodies as indicated by the observations herewith reported. Whereas all of these had a positive skin reaction we have 10 controls which showed no such reaction. There was no correlation between the size of the skin reaction and the time that had elapsed from the date of the typhus fever infection

TABLE II.

Type of Case	Age	Reaction to Rickettsia Suspension	Reaction to Proteus X19 Filtrate	Interval between Infection and Test	Size of Reaction cm.
Recovered	19	++	—	2½ yrs.	4
"	40	++	—	1 yr.	2
"	46	++	—	4 "	2½
"	41	+	—	3 "	1½
"	55	+	—	4 "	1
"	38	++	—	4 "	3
"	55	+	—	4 "	1
"	46	+	—	5 "	1
* " T.A.O.	53	+	—	6 mos.	1
* " T.A.O.	47	+	—	8 yrs.	1
Convalescent Typhus	38	++	—	1 week	2½

*Had symptoms of thrombo-angiitis obliterans.

and the time of the test. Although this series is a small one, these findings suggest that the skin sensitivity lasts a considerable length of time. Whether or not this constitutes an evidence of a latent infection with Rickettsia bodies must as yet be determined.

8083 P

Conditions Determining Melanosis of a Virus-Induced Rabbit Papilloma (Shope).

J. W. BEARD. (Introduced by Peyton Rous.)

From the Rockefeller Institute for Medical Research, New York City.

The Shope papilloma, a growth caused by a virus[1] and having the immediate characters of a tumor,[2] is frequently colored gray, brown or black with melanin.[1] Bits of the proliferating tissue when implanted in the leg muscles and viscera of the host grow vigorously, and portions of the resulting mass are often melanotic, and occasionally it is darkly pigmented everywhere.[2] This paper is concerned with the conditions determining the pigmentation.

The possibility that certain strains of the causative virus determine the pigmentation was investigated first. Two virus suspensions were prepared from glycerinated fragments of 2 peritoneal autotransplants in a wild rabbit. One of the growths was coal black, the other unpigmented. The suspensions were inoculated by tattooing them into numerous spots in the shaved skin of the sides

[1] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

[2] Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934 **60**, 701.

of 2 cottontail rabbits, 4 brown-gray or agouti rabbits of domestic breed and 2 of the Dutch variety (show type). These last had white hair on the anterior half of the body, black hair on the posterior, and the inoculations were made into both regions. All of the papillomas that arose in the "white" regions were devoid of pigment, whereas all those in the "black" were gray or sooty, irrespective of the derivation of the inoculum. The results in the other animals corroborated these findings.

In the animals just considered, as well as in many others inoculated with other strains of the virus, melanotic papillomas developed only where the hair was pigmented. This distribution of the pigmented growths corresponded absolutely with that of melanoblasts or potential melanoblasts in the skin of the rabbit. That melanotic papillomas arise only where such cells are present was shown even more clearly in frozen sections of early stages of growths induced in the skin of the ear. The staining was with dopa (3,4-dioxyphenylalanin).[3] When the first epithelial thickening took place, melanoblasts similar to those nearby in the unaffected epidermis proliferated in the basal part of the papillomatous epithelium and often became extraordinarily abundant, and black with pigment. When the papilloma developed where no melanoblasts were present, none appeared in it later. They were never observed in the growths of albino rabbits. Where they were present to begin with in certain parts of large papillomatous areas, they remained localized to these parts, the rest of the growth never becoming pigmented. Melanoblasts were not seen in the corium, but chromatophores laden with melanin made their appearance under old pigmented growths. The implantation in the viscera of bits of the wholly pigmented growths gave rise usually to nodules that were only partially melanotic.

All of the papillomas have the same histology, except for the presence of melanoblasts and melanin in some of them. They are epithelial growths with a pigmentation which is secondary to the epithelial proliferation. Histologically, they are wholly unlike the melanomas composed primarily of melanoblasts or related cells. In the papilloma the relation of the melanoblasts to the epidermal cells is similar to that found occasionally in regenerating epidermis, in psoriasis, molluscum contagiosum and in the papillomas of man and other animals. No growths composed predominantly of melanoblasts were encountered, nor was evidence obtained of any direct effect of the virus upon them.

[3] Laidlaw, George F., and Blackberg, Solon N., *Am. J. Path.*, 1932, **8**, 491.

In 7 of 10 domestic rabbits kept for 7 months or more, cancers arose in pigmented Shope papillomas.[4] Some of the cancers were papillomatous, but none of their cells reacted with dopa.

From these observations it is plain that the pigmentation of Shope papillomas is not determined by the strain of virus inducing the growths but by the epidermis upon which it acts. Where melanoblasts are present pigmented growths arise because these cells become involved in the pathological process though not themselves affected by the virus.

8084 P

Colony Morphology of Tubercle Bacilli. II. Influence of pH of Culture Medium on Colony Morphology and Virulence.

KENNETH C. SMITHBURN.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

Extensive studies have been made with reference to the range of pH in which tubercle bacilli will grow and the changes in pH which take place during growth on fluid medium. These studies have been reviewed by Wells and Long.[1] Recently Birkhaug[2] has studied the changes in pH induced in fluid medium during growth of R and S forms of avian and mammalian tubercle bacilli. However, the influence of pH on colony morphology of Mycobacteria has been less extensively investigated. Steenken, Oatway, and Petroff[3] found that R colonies of degraded virulence were characteristic of the growth at pH 6.1 on either Petroff's or Calmette's medium; whereas Petroff's medium at pH 7.2 supported the growth of S variants of greater virulence. Recently it was observed in this laboratory[4] that by adjusting the pH of Corper's medium to various values, three principal types of colonies could be obtained: rough, smooth, and intermediate. The present report is an extension of the latter work.

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[4] Rous, Peyton, and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 578.

[1] Wells, H. G., and Long, E. R., The Chemistry of Tuberculosis, 1932, The Williams and Wilkins Company.

[2] Birkhaug, K. E., *Ann. Inst. Pasteur*, 1935, **54**, 19.

[3] Steenken, W., Jr., Oatway, W. H., Jr., and Petroff, S. A., *J. Exp. Med.*, 1934, **60**, 515.

[4] Smithburn, K. C., *J. Exp. Med.*, 1935, **61**, 395.

Twenty-five strains of tubercle bacilli were used in the experiments. Included in these were: R and S variants of an avian strain, a freshly isolated avian strain, 11 bovine, and 11 human strains. Ten of the human strains were isolated within the last 16 months. Corper's glycerinated egg-yolk medium with Congo Red was used throughout. 450 cc. lots of the latter were adjusted to various pH values between 6.0 and 7.2 by intervals of 0.2 pH, the pH determinations being done by the glass electrode method through the courtesy of Dr. D. A. MacInnes. Phosphate buffer (M/1.5 KH_2PO_4 or M/1.5 Na_2HPO_4) was added in the quantity just sufficient to bring to the desired pH value. After adjusting the pH, the medium was placed in tubes and inspissated in the usual manner. In the second and third generations of cultures the medium was adjusted only to pH 6.0, 6.4, 6.8, and 7.2.

Stock cultures of each of the 25 strains were suspended in saline and 3 tubes of medium of each pH were seeded from each suspension. Therefore, in the first generation each of the 24 tubes of each strain of organisms was seeded from a single suspension. In the second and third generations the transfers were made in the same manner except that organisms grown at any one pH value were seeded on medium of the same pH value. Colony selection was not done.

All cultures were examined with the binocular dissecting microscope after 3 and 4 or 5 weeks' incubation at 37°C. The relative amount of growth and colony characteristics of each culture were recorded.

Sharp differences in colony morphology at various pH values were noted in each of 3 avian, 11 bovine, and 4 human strains in the first generation. In the second generation the remaining 7 human strains showed the same differences as were noticed in the 4 human strains of the first generation. These differences may be summarized as follows:

1. Avian Strains. Rough colonies only at pH 6.0 and 6.2. All other cultures showed smooth, creamy colonies. This was true of the avian R as well as the avian S and freshly isolated avian strains. The avian S and fresh avian strains also showed smooth colonies at pH 6.0 in the third generation.

2. Bovine Strains. Rough colonies at pH 6.0 and 6.2. Smooth colonies from pH 6.4-6.8. These were rounded, shiny and non-granular. Intermediate, flat, spreading, finely granular, semi-transparent, "stippled" colonies from pH 7.0-7.4.

3. Human Strains. Rough colonies at pH 6.0 and 6.2. Smooth colonies at pH 6.4 and in a few instances at pH 6.6. These were also rounded, shiny and non-granular. Intermediate colonies from pH 6.8-7.4. These were similar in appearance to the bovine intermediate forms.

The data above indicate the dominant colony forms at the various pH values. When other colony forms appeared than those indicated, they were in the minority.

Total growth varied remarkably at the different pH values but showed no appreciable variation in the human, bovine or avian types. Growth was most vigorous at pH 6.0 and 6.2 and in general less vigorous the more alkaline the medium.

Animals have been inoculated with these colony variants derived from a freshly isolated human strain, a highly virulent bovine strain, and a bovine strain of very low virulence. From the latter experiments it has been found that the rough colonies are of least virulence, the smooth of greatest virulence, and the intermediate colonies are of intermediate virulence. Smooth colonies derived from the bovine strain of low virulence are apparently not more virulent than the undissociated culture from which they were derived.

Since all other variables were avoided, save that of hydrogen ion concentration of the standard medium, these experiments demonstrate the rôle which one factor may play in determining the colony morphology and virulence of tubercle bacilli.

8085 P

Histological Observations on Resistance to Transplantable Leukemia in Immunized Mice.

J. S. POTTER AND M. D. FINDLEY. (Introduced by E. C. MacDowell.)

From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, L. I., N. Y.

Recently it has been demonstrated that immunization to transplantable lymphatic leukemia in mice can be actively induced by suitable injection of dilute doses of leukemic cells,[1] by injection of nor-

[1] MacDowell, E. C., Taylor, M. J., and Potter, J. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 84.

mal lymphoid cells,[2] and of embryo skin.[3] Naturally susceptible mice so treated are resistant to injections of normally lethal doses of leukemic cells at subsequent inoculations. This paper records the results of preliminary observations on the fate of malignant lymphoid cells inoculated into immunized mice.

A group of 19 mice of strain C58, immunized to line I transplantable lymphatic leukemia by dilute doses of line I cells as already described,[1] were killed at intervals following inoculation of a massive dose; 1 at 13½ hours, 1 at 1 day, 3 at 2 days, 7 at 3 days, 2 at 4 days, 1 at 5 days, 2 at 6 days, and 2 at 7 days. Unimmunized mice inoculated with this line die at 3½ to 4 days following inoculation. The development of lesions of this line of leukemic cells in hosts of strain C58 as previously described[4] forms a basis for comparison with the behavior of these leukemic cells in immunized hosts.

Examination of the tissues from the immunized animals shows that at first the inoculated cells continue to proliferate. Lesions may appear in the same areas as in unimmunized mice but never become so large. The percent of cells in division is low (0.3%-1.0%) as compared with active lesions at all stages in unimmunized mice (4%-9%) inoculated with line I.

Degeneration of the infiltrating cells usually takes place within the first 4 days. In only 2 animals out of the 14 killed after the second day were active lesions found without necrotic cells, but in these 2 animals necrotic lesions were also found, indicating that recovery was in progress. The process of degeneration of lesions apparently follows a regular sequence. The first indication is the degeneration of single cells in the peripheral margin of the lesion, and the degeneration spreads rapidly until chromophilic debris occupies the supporting stroma. At this time the host phagocytes appear and the debris promptly disappears. The remaining stroma may be invaded by host lymphoid cells, which appear at the site of the lesion for the first time at this stage, but soon disappear. In only one case was lymphoid hyperplasia associated with invasion by host cells. By the 6th and 7th day no evidence of the inoculation can be found.

The same stages of degeneration observed in actively immunized mice have also been observed in a naturally resistant strain, Sto-Li, following inoculation with line I.

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2 Rhoads, C. P., and Miller, D. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 817.

3 MacDowell, E. C., in press.

4 Potter, J. S., and Richter, M. N., *Arch. Path.*, 1933, **15**, 198.

Immunity to transplantable lymphatic leukemia is apparently not the result of blockade or invasion of lesions by normal host lymphocytes as has been supposed in resistance to other types of neoplasms,[5] since the injected cells are necrotic before the mobilization of host cells.

8086 P

Influence of Thyroid Administration on Creatin Metabolism in Myxedema of Adults.

EPHRAIM SHORR, HENRY B. RICHARDSON AND JAMES S. MANSFIELD.

From the New York Hospital, the Department of Medicine of the Cornell University Medical College, and the Russell Sage Institute of Pathology, New York City.

Little is known of the creatin metabolism of adult myxedema other than the absence of spontaneous creatinuria. In children with cretinism or hypothyroidism, the physiological creatinuria of childhood is absent.[1, 2] When thyroid substance is given to such children, large amounts of creatin appear in the urine.[1, 2] This effect has been interpreted as a restoration of the normal creatinuria of childhood. It is not known, however, how long the creatinuria persists with continued administration of thyroid substance in amounts sufficient to maintain a normal basal metabolism.

We have studied the creatin metabolism of 2 typical cases of spontaneous adult myxedema. Their diet was creatin-creatinin-free, and adequate in calories and protein. The ability to retain ingested creatin (1.32 gm.) was tested from time to time. After a control period, thyroid substance was administered in amounts sufficient to restore and maintain a normal basal metabolism. The usual improvement in signs and symptoms took place. Six cases of myxedema following total ablation of the thyroid* were studied in the same way, except that no thyroid substance was given. Our findings in spontaneous myxedema are given in Table I and Chart 1.

Results. In agreement with other workers, we found no creatinuria in spontaneous adult myxedema. Creatinuria was also ab-

[5] Murphy, J. B., Monographs of the Rockefeller Inst. for Med. Res., 21, 1926. Lumsden, Thomas, *Am. J. Cancer*, 1931, **15**, 563.

[1] Beumer, H., and Iseke, C., *Berlin. Klin. Wchnschr.*, 1920, **57**, 178.

[2] Poncher, H. G., Visscher, M. B., and Woodward, H., *J. Am. Med. Assn.*, 1934, **102**, 1132.

* We are indebted to Dr. Herrman L. Blumgart of Boston for these cases.

TABLE I.

Creatin Metabolism in Adult Myxedema Following Thyroid Extract.
Case I: K.B. (F), Age 50.

Date 1935	——Urinary Creatin—— Spontaneous gm.	After Creatin 1.32 gm. gm.	Creatin Tolerance %	B.M.R. %	Thyroid Extract
2/24-26	0.000	0.190	81	—21	none
3/17-19	0.108	0.557	55	—30	gr. ½ (3/7-14) gr. 1 (3/14-19)
3/31-4/2	0.413	1.050	33	—11	gr. 1 (3/19-21) gr. 2 (3/21-)
4/22-24	0.060	0.634	43	—1	gr. 2 (3/21-)

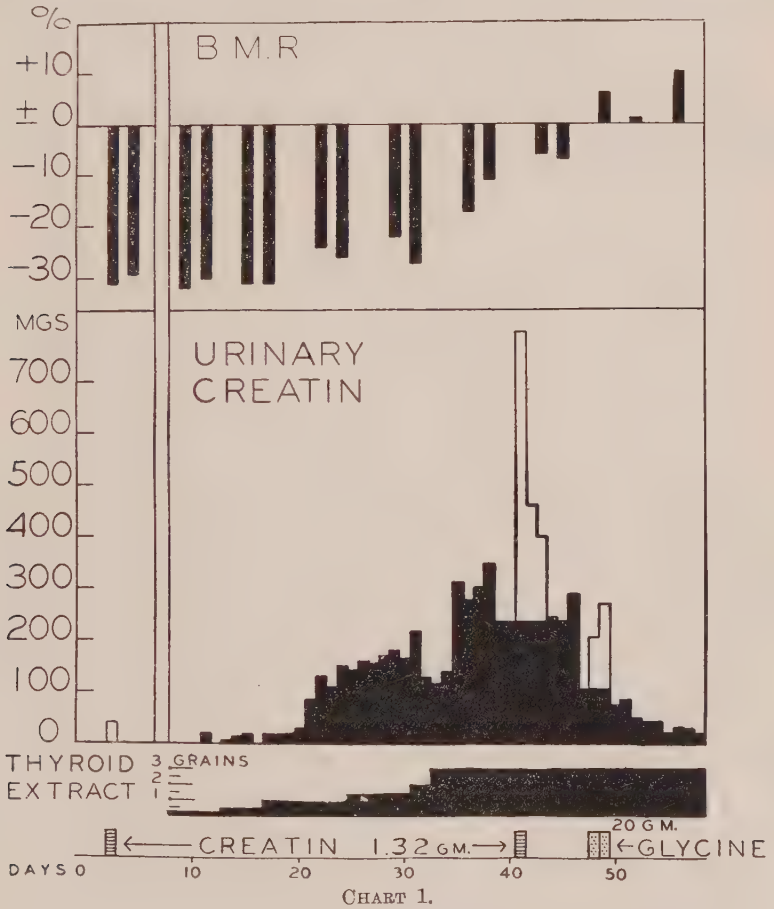

CHART 1.

Creatin Metabolism in Adult Myxedema Following Thyroid Extract.
Case 2: M.R. (F) Age 62.

Shaded areas indicate spontaneous creatinuria. Unshaded areas indicate "extra creatin" following administration of creatin and glycine.

sent in myxedema following total ablation of the thyroid. In both groups the retention of ingested creatin was normal. In spontaneous myxedema small doses of thyroid substance caused a pronounced creatinuria and a diminished tolerance to ingested creatin. This was observed before any elevation in the basal metabolism. In one case, at the height of the creatinuria, administration of glycine resulted in an excretion of extra creatin. This is comparable to our findings in Graves' disease.[3] With continued thyroid administration, with the basal metabolism at normal levels, the creatin excretion gradually fell, and approached normal values. Apparently there is an eventual adjustment to thyroid administration. Whether a similar adjustment takes place in hypothyroid children cannot be answered from the data in the literature. This sensitivity of the creatin metabolism of adult myxedema to small doses of thyroid substance at low levels of basal metabolism offers an additional criterion for diagnosis.

8087 P

Effect of 4-6 Dinitro-o-Cresol on Yeast Respiration.*

JOHN FIELD, 2ND.

From the Department of Physiology, Stanford University.

Field, Martin and Field[1-4] have shown that stimulation or inhibition of yeast respiration by 2-4, 2-5 or 2-6 dinitrophenol or by p- or m-nitrophenol is determined by the concentration of the undissociated form. It is well known that other nitrated phenols are direct stimulants of cellular metabolism, one of the most active of these being 4-6 dinitro-o-cresol (hereinafter DOC) which has been

[3] Shorr, E., Richardson, H. B., and Wolff, H. G., *J. Clin. Invest.*, 1933, **12**, 966.

* Supported by grants from the Committee on Scientific Research of the American Medical Association and from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

[1] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[2] Field, J., 2nd, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, 1934, **4**, 405.

[3] Field, J., 2nd, Martin, A. W., and Field, S. M., *J. Pharmacol. and Exp. Therap.*, 1935, **53**, 314.

[4] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1043.

studied by Dodds and his co-workers.[5-9] The experiments reported below show that DOC, like the other nitrated phenols mentioned, affects yeast respiration only in the undissociated form. The pure culture of *Saccharomyces cerevisiae* and the experimental procedure were the same as in our previous work.[1, 2, 3]

It is shown in Table I that in doses of DOC evoking optimal stimulation of yeast respiration the concentration of the undissociated form is quite constant over a wide pH range, while the total concentration causing optimal stimulation varies about 80-fold between pH 5.4 and pH 7.8. The table represents the summarized data from 36 experiments.

TABLE I.

Constancy of the Concentration of Undissociated DOC Evoking Optimal Stimulation of Yeast Respiration at Various pH Levels. The Dissociation Constant of DOC Was Found to Be 1×10^{-5}.

pH	Total conc'n DOC mg. %	Total conc'n DOC molar	Molar conc'n undissociated DOC	O_2 consumption of control in mm^3 per 10^8 cells per hr.	O_2 consumption with optimal dose of DOC in mm^3 per 10^8 cells per hr.	Rate of O_2 consumption at opt. dosage control = 100
5.8	0.6	3.06×10^{-5}	8.71×10^{-6}	33.3	54.5	164
6.0	2.0	1.02×10^{-4}	9.26×10^{-6}	30.7	46.7	152
6.8	10.0	5.1×10^{-4}	7.96×10^{-6}	33.0	52.6	159
7.8	40.0	2.04×10^{-3}	3.22×10^{-6}	15.9	21.9	138

It was also found that change in pH during a run modifies or reverses the effect of DOC on yeast respiration in a manner most directly explicable by changes in concentration of the undissociated form, and that the action of the drug develops rapidly, being comparable in this respect to the action of 2-4 and 2-6 dinitrophenol (Field, Martin and Field[2, 3]). Study of the increased respiration evoked by a dose (total) of 0.1 mg. % DOC at pH 5.2 showed that in 3 hours the extra oxygen consumption evoked by the drug was 14 times the amount required for complete oxidation of the DOC present, which is clear evidence that DOC serves to increase the rate of oxidative metabolism of yeast in catalytic fashion rather than merely as another fuel.

Conclusions. 4-6 dinitro-o-cresol affects the respiration of yeast only in the undissociated form. Inhibition is reversible by increase

[5] Dodds, E. C., and Pope, W. J., *Lancet*, 1933, **2**, 352.
[6] Dodds, E. C., and Robertson, J. D., *Lancet*, 1933, **2**, 1137.
[7] Dodds, E. C., and Robertson, J. D., *Lancet*, 1933, **2**, 1197.
[8] Dodds, E. C., and Greville, G. D., *Nature*, 1933, **132**, 966.
[9] Dodds, E. C., and Greville, G. D., *Lancet*, 1934, **1**, 398.

in pH within wide limits of concentration and exposure time. The drug acts on enzyme systems located in the periphery of the yeast cell and the action is catalytic rather than stoichiometric.

8088 C

Physiological Activity of Some Catechol Derivatives.

MICHAEL G. MULINOS AND RAYMOND L. OSBORNE.

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University.

Muhlmann,[1] and later Dakin[2] determined that catechol and certain of its derivatives that resemble epinephrine in their skeletal structure were capable of raising the blood pressure of the anesthetized rabbit. Barger and Dale[3] found that catechol raised the blood pressure of the decapitated cat, showing that this drug acts peripherally to the vasomotor center. They claimed also that catechol did not dilate the pupil of the intact animal, but did cause contraction of the isolated virgin cat uterus, and drew the conclusion that catechol raises the blood pressure by direct muscle stimulation, rather than by sympathetic imitation.

We have confirmed their results, on blood pressure and pupil, but have determined further that catechol depresses the uterus and intestine of many animals. On the basis of these findings, it occurred to us to test anew the suggestion of Dakin that epinephrine owes much of its typical sympathomimicity to the catechol group.

Our results show that catechol, ethylcatechol, acetocatechol, and chloracetocatechol possess sympathomimetic powers (Table I). In this, inhibitory effects predominate, so that when an organ contains but one type of sympathetic innervation, as in the dilator pupili, the nictitating membrane, or the intestine, that action is brought out by these drugs. On the other hand, when both motor and inhibitor fibers of the sympathetic are present in the same organ, as in the blood vessels and uterus, the inhibitory action predominates, although the action of catechol is not reversed after ergotoxine. In epinephrine, the motor effects are much more in evidence than the inhibitor, but other non-catechol containing amines

[1] Muhlmann, *Deutsch. Med. Wchnsch.*, 1896, **22**, 409.

[2] Dakin, *Roy. Soc. Proc. B.*, 1905, **76**, 498.

[3] Barger and Dale, *J. Physiol.*, 1910, **41**, 19.

TABLE I.

Animal and Organ	Chloracetylcatechol	Acetocatechol	Ethyl catechol	Catechol	Epinephrine	Ephedrine
Uterus						
Virgin Cat	—(16	—(5	—(3)	—(12)	—(10)	0(2)
						—(4)
Pregnant Cat	+(4)	—(3)	—(3)	—(3)		—(6)
	0(25)			0(6)	+(27)	
Rabbit	—(21)	—(3)	—(3)	—(8)	+(9)	—(3)
				0(2)		+(3) 0(3)
Virgin Guinea Pig	—(6)	—(3)	—(3)	—(2)	—(4)	
Small Intestine						
Cat	—(20)	—(4)	—(4)	—(11)	—(14)	—(7)
						0(8)
Rabbit	—(30)	—(5)	—(4)	—(13)	—(13)	—(7)
						+(4)
Monkey	—(46)			—(21)	—(15)	—(10)
						+(2) 0(9)
Blood Pressure (16 animals)						
Intact Cat	+(44)	+(6)	+(6)	+(13)	+(26)	+(4)
Pithed Cat	—(24)			+(5)	+(16)	+(3)
Pupil						
Cat, Rabbit	dil.	0	0	0	dil.	dil.
Excised Frog; Beef	dil.	0	0	0	dil.	dil.
Blood Sugar						
Rabbit	+(10)			+(6)	+(75)	+(6)

\+ means stimulation.
— means depression.
0 means no effect. A blank means not tried. The numbers refer to total injections on 20 cats; 12 rabbits; 71 strips of intestine; 44 strips of uterus.

such as ephedrine seem incapable of strong inhibitory activity, especially in the presence of motor fibers.

Chloracetylcatechol is the most powerfully sympathomimetic drug of our series. In it a chloride molecule has replaced the usual amino group ordinarily associated with sympathomimetic activity. Chloracetylcatechol raises the blood pressure by medullary stimulation, for there is no rise but a fall after decapitation. Catechol retains its vaso-pressor powers, even after decapitation and double adrenalectomy. Chloracetylcatechol alone, of these derivatives, causes pupilo dilation, even after pithing and double adrenalectomy. The blood sugar of unanesthetized rabbits is raised. It depresses the isolated intestine and virgin cat uterus, but has no effect on, or occasionally stimulates the pregnant cat uterus.

In view of these findings for 3,4-dihydroxy-phenylchlormethyl-ketone (chloracetylcatechol), and those of Schaumann[4] for 3,4-dihydroxy-ephedrine; of Hartung,[5] etc., for 3,4-dihydroxy-phenyl-l-amino-2-propanol; of Tiffeneau[6] for 3,4-dihydroxy-benzylamine;

of Barger and Dale[3, 9] for epinephrine and for β-3,4-dihydroxy-phenyl-ethanolamine; of Pyman[7] for 3,4-dihydroxy-phenyl-ethylmethylamine; and of Alles[8] for β-3,4-dihydroxy-phenyl-isopropylamine and β-3,4-dihydroxy-phenyl-ethylamine, we agree with Dakin that the catechol nucleus is in great part responsible for the typical sympathomimicity of epinephrine. The 3,4-position of the phenyl-hydroxyls; a 2 carbon side chain; a beta carbon hydroxylated; an alpha carbon hydrogen substituted by some indifferent molecule, preferably an amine; and where all of these are present, the levorotatory isomer, are contributory factors in the formation of a typically sympathomimetic drug.

8089 C

A Poliomyelitis Antiserum.*

JOHN A. TOOMEY.

From the Department of Pediatrics, Western Reserve University, and the Division of Contagious Diseases, City Hospital, Cleveland, Ohio.

A normal sheep was bled, and no neutralizing antibodies for poliomyelitis virus were found in the blood serum in 1:1 dilutions. The animal was then injected as follows: September 8, 12, 17, and 24, 1934, 10 cc. of a suspension of equal parts of 2% poliomyelitis virus and P.C.B. filtrate intradermally;[1] October 1st and 8th, 30 cc. of a suspension of equal parts of a 2% virus combined with P.C.B. filtrate subcutaneously; October 13th, 40 cc. of a suspension made up of equal parts of a 5% virus combined with P.C.B. filtrate subcutaneously; October 23rd, November 8th and 20th, and December 8th, 50 cc. of a suspension made up of equal parts of 5% virus combined with P.C.B. filtrate subcutaneously and intramuscularly. On September 13th, there was some local induration about the injection, and on September 15th, there was a small localized abscess.

[4] Schaumann, *Arch. Exp. Path. u. Pharm.*, 1931, **160**, 127.

[5] Hartung, Munch, Miller and Crossly, *J. Am. Chem. Soc.*, 1931, **53**, 4149.

[6] Tiffeneau, *Ch. Richet Festschritt*, 1912, 399.

[7] Pyman, F., *J. Chem. Soc.*, 1910, **97**, 266.

[8] Alles, G., *J. Pharm. and Exp. Therap.*, 1933, **47**, 339.

[9] Barger, Some Applications of Organic Chemistry to Biology and Medicine, 1930, 80.

* Expenses defrayed in part by a grant from the Marion R. Spellman Fund, The Cleveland Foundation.

[1] Toomey, John A., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 869.

Although there was still evidence of local induration about the last injection, the animal was bled on November 20th and the serum tested. It was found that this serum, obtained only 12 days after an injection of the virus and filtrate combination, acted as an accelerator, since the disease was produced in the test animal in 3 days, and in the control in 7 days. That the serum was tested too soon after the injection was obvious from later results. The animal was not bled again until January 18, 1935, at a time when no external evidence of reaction was found in the skin or in the area over the muscles where the injections had been made.

The sheep serum was inactivated at 56°C. for 1 hour. In these experiments, the injections were all made intracerebrally under complete ether anesthesia.

Set I. Monkey No. 406, injected with 0.5 cc. of solution made up of 0.25 cc. of 2% virus plus 0.25 cc. of whole sheep serum: no effect. †Monkey No. 407, injected with 0.5 cc. of solution made up of 0.25 cc. of 2% virus plus 0.25 cc. of saline: tremor and furred, 5th day; quadriplegia, 7th day.

Set II. Monkey No. 408, 0.5 cc. of solution made up of 0.25 cc. of 2% virus plus 0.25 cc. of ½ sheep serum in saline: no effect. †Monkey No. 404, same as No. 407: furring 4th day; quadriplegia 7th day. Monkey No. 409, 0.5 cc. of solution made up of 0.25 cc. of 2% virus plus 0.25 cc. of ¼ sheep serum in saline: no effect. †Monkey No. 401, same as No. 407: furred, not active 5th day; quadriplegia, 7th day. Monkey No. 410, 0.5 cc. of solution made up of 0.25 cc. of 2% virus plus 0.25 cc. of ⅛ sheep serum in saline: no effect.

Set III. Monkey No. 412, 0.25 cc. of solution made up of 0.125 cc. of 2% virus plus 0.125 cc. of 1/16 sheep serum in saline: no effect. †Monkey No. 411, 0.25 cc. of solution made up of 0.125 cc. of 2% virus plus 0.125 cc. of saline: inactive, furred, clumsy 7th day; paralysis 10th day. Monkey No. 413, 0.25 cc. of solution made up of 0.125 cc. of 2% virus plus 0.125 cc. of 1/32 sheep serum in saline: no effect. †Monkey No. 402, same as No. 411: paresis 6th day; quadriplegia 7th day. Monkey No. 414, 0.25 cc. of solution made up of 0.125 cc. of 2% virus plus 0.125 cc. of 1/64 sheep serum: no effect save some slight furring on the 6th day. †Monkey No. 289, same as No. 411: sick, furred, anorexia 9th day; paralyzed 10th day.

Set IV. †Monkey No. 415, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of saline: furred and tremor 6th day;

† Control.

quadriplegia 7th day. Monkey No. 416, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of 1/128 sheep serum: furred 16th day; tremor and paralysis 17th day. This animal, although protected, died. Before injection, however, it was thin and scrawny and had been losing weight. Autopsy showed a marked gastro-enteritis with a matting together of the lower portion of the ileum and numerous tuberculous enlargements of the mesenteric and intestinal glands. Several tubercles were found in the lung. Monkey No. 422, was injected later with the same dose of material that Monkey No. 416 had received and did not contract poliomyelitis. Monkey No. 421, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of 1/256 sheep serum: no effect.

Set V. †Monkey No. 423, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of saline: furred 3rd day; quadriplegia 6th day. Monkey No. 424, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of 1/512 sheep serum in saline: furred 3rd day; no paresis or paralysis. Monkey No. 425, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of 1/1024 sheep serum in saline: furred 2nd day; no paresis or paralysis.

Set VI. †Monkey No. 450, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of saline: furred 4th day; quadriplegia 7th day. Monkey No. 448, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of 1/1536 sheep serum in saline: furred 8th day; paralysis of the muscles of the left leg 9th day; paralysis of some of the muscles of the right leg 10th day; paralysis of some of the muscles of the left arm 11th day; recovered with residual muscle paralyses of both legs. Monkey No. 447, 0.3 cc. of solution made up of 0.15 cc. of 2% virus plus 0.15 cc. of 1/2048 sheep serum in saline: furred 5th day; quadriplegia 7th day.

Animals No. 422 and No. 448 were observed for over one month, while all the other surviving animals were observed for over 3 months.

Thus in 3 months a potent unconcentrated antipoliomyelitic sheep serum was produced by injecting a sheep with crude virus (7 gm.) combined with P.C.B. filtrate. This serum neutralized the virus completely in a dilution of 1:1024.

8090 C

Acute Splenic Tumor Produced by Non-Bacterial Antigens.

ARNOLD RICE RICH. (Introduced by J. Howard Brown.)

From the Department of Pathology and Bacteriology, The Johns Hopkins Medical School, Baltimore.

It is well known that during the course of bacterial infections the spleen undergoes a change designated by the term "acute splenic tumor". The significance of this change has never been clearly understood. The essential microscopic characteristic of the condition is an increase in mononuclear cells which have not the appearance of ordinary lymphocytes. These cells appear first in the margins of the Malpighian bodies and spread throughout the pulp in advanced cases. Some are definitely plasma cells, but the majority have large vesicular nuclei and abundant basophilic cytoplasm, and there has never been any general agreement as to their nature or function. Some have described them as macrophages, some as lymphoblastic cells, but most writers have regarded them as young myeloid cells and have interpreted their presence as representing an activity of the spleen in the manufacture of granulocytes under the stimulus of the infection.

Although young myeloid cells may be found in the spleen (as in the blood) in bacterial infections, and although myeloid cell formation may undoubtedly occur in the spleen during the course of some infections, no acceptable evidence has ever been brought forth that the great body of newly formed mononuclear cells characteristic of acute splenic tumor are myeloid cells. They are non-granular cells, and the writer has never been able to observe any indication of their transformation into granulocytes. In view of the large amount of evidence that the spleen plays an important rôle in antibody production, and since acute splenic tumor and antibody production are factors that are common to bacterial infections of widely different nature, it has been suggested from time to time that acute splenic tumor may be an expression of the formation of protective substances active against the infecting bacteria and their products. Since experiments with bacteria and toxins introduce a variety of complicating factors, the present experiments were carried out to determine whether the essential characteristics of acute splenic tumor could be produced simply by inciting the formation of antibody in the absence of bacterial infection or bacterial substances or products.

Several investigators have examined the spleen histologically dur-

ing the course of experiments in which non-bacterial foreign protein was injected for various purposes but, so far as the writer is aware, none of the descriptions of the splenic changes following the injection of foreign protein can be clearly interpreted as a reproduction of those occurring in acute splenic tumor. Thus Cantacuzène's description of the cellular alterations that he observed[1] is totally different from that in acute splenic tumor; Epstein[2] observed various changes in the Malpighian bodies but described neither the production of the specific basophilic mononuclear cells nor any alteration of the pulp; Kuczynski[3] does describe "lymphoblastic" cells in the spleen of mice injected with or fed upon foreign protein, but the untreated mouse's spleen contains these cells to such a degree that in its normal state it resembles an acute splenic tumor. None of the above investigators studied the spleen from the standpoint of a comparison with acute splenic tumor.

In the present experiments, rabbits of approximately equal weight were used as the experimental animals, and sterile egg white, horse serum, dog serum or guinea pig serum as the antigen. Antibody formation was stimulated by (1) a single intravenous injection of 5.0 cc. or 10.0 cc. of antigen; (2) repeated intravenous injections of 1.0 cc. of antigen; (3) repeated intracutaneous and subcutaneous injections of 0.2 cc. to 1.0 cc. of antigen until sensitization of the Arthus phenomenon type was established.

Fourteen test rabbits and 14 controls were used in the experiments. Half were treated by intravenous injection and half by subcutaneous and intracutaneous injection. As a control for each animal that was injected with serum a rabbit of the same weight was injected in the same manner with normal salt solution. Test and control animals were killed at intervals ranging from 3 days to several weeks after the first injection but always within 3 days of the last injection. The temperatures of all the animals taken just before and the day following each injection remained within normal limits, and no intercurrent infection was found in any case at autopsy.

At autopsy, the average weight of the spleens of the animals in which antibody formation had been induced was 60% greater than that of the spleens of the controls, and the microscopic appearance of the spleens of all of the animals that received foreign protein was strikingly different from that of the spleens of the controls. In the

[1] Cantacuzène, J., *Ann. Inst. Past.*, 1908, **22**, 54.

[2] Epstein, E., *Virch. Arch.*, 1929, **273**, 89.

[3] Kuczynski, M. H., *Virch. Arch.*, 1922, **239**, 185.

test animals killed during the first few days there was a marked mitotic proliferation and enlargement of the mononuclear cells in the marginal zones of the Malpighian bodies. The nuclei became greatly enlarged and the cells developed an abundant basophilic cytoplasm and became identical in appearance with the specific large mononuclear cells of the acute splenic tumor of infection. There was no suggestion of a transformation of these cells into granulocytes in any of the animals. A precisely similar change constitutes the early stage of the acute splenic tumor occurring in bacterial infections. As the process advances, the specific mononuclear cells spread out into the pulp of the spleen of the antibody-forming rabbits precisely as in the acute splenic tumor of infection, and the histological picture becomes quite like that in a well developed acute splenic tumor.

Details of further studies dealing with the nature and characteristics of the specific cells as revealed by vital stains and motion pictures of tissue cultures, with the relation between the antibody titre and the degree of proliferation of these cells, with the lack of correlation between this reaction and granulocyte production in the spleen, and with a similar mononuclear cell reaction occurring in lymph nodes during infection and antibody formation will be presented in a separate report, together with a consideration of the literature. Here it is desired to point out only that the stimulus of foreign protein, uncomplicated by bacterial infection or bacterial substances or products, suffices to incite the specific mononuclear cell reaction characteristic of acute splenic tumor.

8091 P

Glycogen Formation After Various Fatty Acids.

HARRY J. DEUEL, JR., JOSEPH S. BUTTS AND LOIS HALLMAN.

From the University of Southern California School of Medicine.

The transformation of the even-chained fatty acids into the acetone bodies has been demonstrated to be a quantitative one.[1] It was shown that such odd-chained fatty acids as propionic, valeric and heptoic did not give rise to appreciable amounts of the acetone

[1] Butts, J. S., Cutler, C. H., Hallman, Lois, and Deuel, H. J., Jr., *J. Biol. Chem.*, 1935, **109**, 597.

bodies. With the exception of the experiments of Ringer[2] on phlorhizinized dogs and the negative results of Eckstein[3] on glycogen formation in the white rat, no experimental evidence is on record regarding the glycogenic ability of the various fatty acids.

In the present tests the sodium salts of propionic, diacetic, butyric, valeric, caproic, heptoic, caprylic and nonylic acids were fed, by stomach tube, to rats previously fasted for 48 hours in doses equivalent to 1 mg. (calculated as acetone) per sq. cm. of body surface. In series 1 the rats were killed 6 hours after the administration of fatty acids: in series 2, seven hours after such administration. The average results on liver glycogen in series 1 are as follows: Control, (10 animals) 0.27% (Range 0.11-0.71); Propionic (9) 1.36% (0.21-1.86); Valeric (10) 0.69% (0.51-1.06); Diacetic (10) 0.18% (0.12-0.22); Butyric (9) 0.30% (0.20-0.46); and Caproic (10) 0.16% (0.09-0.22). In series 2 the results were as follows: Control (9) 0.18% (0.07-0.35); Valeric (10) 0.64% (0.27-1.16); Heptoic (10) 1.02% (0.63-1.54); Nonylic (10) 0.83% (0.43-1.50); Caprylic (10) 0.25% (0.13-0.45).

It is apparent that the odd-chained fatty acids, valeric, heptoic and nonylic, give rise to approximately the same amount of glycogen in the liver as propionic acid. This indicates that the process of beta-oxidation of the odd-chained fatty acids is fairly quantitative. On the other hand, the even-chained fatty acids such as diacetic, butyric, caproic and caprylic are unable to form appreciable amounts of glycogen.

8092 C

Extraction of Estrin from Female Urine After Acidification with Various Acids.

W. KENNETH CUYLER. (Introduced by D. R. McCullagh.)

From the Department of Endocrinology and Metabolism, Cleveland Clinic, Cleveland, Ohio.

In devising a method for the assay of any hormone, practicability and accuracy are the two important factors to be considered. It is more practicable to make assays of estrin from urine than from blood. Estrin assays were made by the method of Mazer and Gold-

[2] Ringer, A. I., *J. Biol. Chem.*, 1912, **12**, 511; 1913, **14**, 43.

[3] Eckstein, H. C., *J. Biol. Chem.*, 1933, **102**, 591.

stein,[1] (a modified Frank and Goldberger technique), who used blood, by that of Kurzrok[2] who used urine, and by that of Robson, MacGregor, *et al.*,[3] by which the urine is hydrolyzed before extraction.

Kurzrok's method,[2] slightly modified, has been in use for clinical purposes in this laboratory for more than a year. A 24-hour specimen of urine is carefully measured. Of the total amount, 750 cc. are used for the assay and if there are only 750 cc. or less, then the total amount is assayed. The urine is put into a flask and is acidified with 15 cc. of glacial acetic acid. Forty or 50 gm. of table salt are added to increase the specific gravity. In addition to making the urine heavier, facilitating the passage of the lighter extracting fluid through the urine, this addition of salt helps to prevent the urine from taking up the extracting fluid. The urine is then extracted continuously with ethyl acetate for 24 hours. The extract is distilled *in vacuo*, the residue is washed with ethyl ether, and 10.5 cc. of sesame oil are added to the ether extract. When the ether has evaporated, the oil which contains the estrin is injected as follows: Six adult, spayed, female rats are used for each assay. It is ascertained by vaginal smears that the rats used had normal estrous cycles before they were spayed and that they are in continuous diestrum after they were spayed. Each of 2 of these rats receives 3.0 cc. of the extract, 2 receive 1.5 cc. each, and 2 receive 0.75 cc. each. The total amount of injected extract is 10.5 cc. Vaginal smears are taken 48 hours after the first injection, and they are continued at intervals of about 8 hours until the end of 72 hours. Diagnosis of positive results is made from estrous and metastrous smears only.

The calculation of the output of estrin for 24 hours is made in terms of rat units according to the following formula:

$$\frac{\text{Total amount of oily extract}}{X} \times \frac{\text{Amount of 24-hour specimen in cc.}}{\text{Amount of urine used for assay}} = \text{Rat Units}$$

The unknown factor represents the smallest amount of the oily extract that produces estrus in 2 rats. By the substitution in this formula of a 24-hour specimen of urine which measures 1 liter, and after having obtained estrous smears by the use of 3.0 cc. of the extract, the following equation is derived:

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1 Mazer, C., and Goldstein, L., Clinical Endocrinology of the Female, Philadelphia, W. B. Saunders & Co., 1932, 155.

2 Kurzrok, R., and Ratner, S., *Am. J. Obst. and Gyn.*, 1932, **23**, 689.

3 Robson, J. M., MacGregor, T. N., Illingworth, R. E., and Steere, N., *Brit. Med. J.*, 1934, No. 3828, 888.

$$\frac{10.5}{3.0} \times \frac{1000}{750} = 4.6 \text{ Rat Units}$$

This result represents the calculated number of rat units of estrin which were extracted from the liter of urine.

Assays for clinical purposes were made more frequently when the presence of hypo-ovarianism was suspected than when hyper-ovarianism was suspected or when patients were thought to be normal. A series of assays which were done on the urine of normal women yielded an average of 6.2 rat units per liter of urine. The range of the estrin content of the urine, which can be measured by the use of the amounts of extract stated above, is from 4.2 to 18.6 rat units per liter of urine. Some of the assays on urine of normal women fell below 4.2 units per liter. Larger amounts of the extract can be used where lower levels are to be measured, but the health of the animals is so much disturbed by the injection of larger quantities of the extract that it makes the value of such a procedure questionable. Since the lower levels of the normal are of the most practical significance, methods which give a higher yield of estrin are desirable.

It is known from the work of others,[4, 5] and from work done in this laboratory,[6] that varying results are obtained in the extraction of active ovarian and testicular principles from urine after it has been acidified with different acids.

This paper presents the results which were obtained by the assay of female stock urine after acidification with various acids. The assays were done according to the modification of Kurzrok's method described above. Commercial sulphuric and hydrochloric acid, crystalline trichloracetic acid, and powdered tartaric acid were used. Glacial acetic acid was used as a control since it had been used to acidify the urine for the assays for clinical purposes and for the assays of the normal controls.

The urine used for the assays on which this paper is based was obtained from the routine clinical laboratories. The amounts of urine obtained at one time varied from 4 to 18 liters. Since it was extracted and assayed as it came to the laboratory, it was necessary to have a control acid for each lot of urine. In order to maintain a standard for the clinical and control assays, glacial acetic acid was used as the control.

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[4] Adler, A. A., *Nature*, 1934, **133**, 789.

[5] Harrow, B., and Sherwin, C. P., The Chemistry of the Hormones, Baltimore, Williams & Wilkins, 1934.

[6] Cuyler, W. K., unpublished results.

Alkalinity of the urine varied considerably. Amounts of the various acids were chosen which would render the urine definitely acid in every case.

The acids were used in the following amounts: (1) Sulphuric acid, 50 cc.; (2) Hydrochloric acid, 50 cc.; (3) Glacial acetic acid, 50 cc.; (4) Trichloracetic acid, 15 gm.; (5) Tartaric acid, 15 gm.

Considerable experimentation was done in order to ascertain whether or not an amount of acid sufficient to acidify the urine or a great excess of acid had any effect upon the results of the assay. It was found that 50 cc. or gm. of an acid in excess of the point where the urine became acid to litmus paper had no effect upon the amount of estrogenic substance which might be extracted.

For the work reported in this paper, an arbitrary average of one liter was taken to represent a 24-hour specimen of urine and 750 cc. of this was extracted for each assay, as is done in the clinical assay.

The following facts may assist the reader in correlating the data to be presented. When estrous smears are obtained after the injection of 3.0 cc. of the oily extract, the calculated number of rat units of estrin in that extract are 4.6; when estrous smears follow the injection of 1.5 cc., the rat units are calculated to be 9.3; when estrous smears occur after the injection of 0.75 cc., the number of rat units is calculated to be 18.6.

Although Robson, MacGregor, *et al.*,[3] used sulphuric acid in their hydrolysis method, its use gave the poorest results in our tests. This acid was used for the acidification in 15 urine assays; no estrous smears were found in any of the 90 rats which were injected, and only 4 of this number showed pro-estrous smears. These smears appeared in rats which received the largest amount of extract, or 3.0 cc. Hydrochloric acid was found to be only slightly more effective than sulphuric acid as an acidifying agent. Seventeen assays were done with the use of this acid; only 5 of the 102 rats injected showed estrous smears, and only one rat showed a pro-estrous smear. These smears occurred in rats which received 3.0 cc. of the extract.

Twenty-four assays were made when glacial acetic acid was used for acidification. A total of 144 rats were injected; estrous smears were found in 41 of the 48 injected with 3.0 cc. of the extract, and in only 3 of 48 rats injected with 1.5 cc. Pro-estrous smears were found in 3 of the rats injected with 3.0 cc., and in only one rat injected with 1.5 cc. None of the 48 rats injected with 0.75 cc. of the extract showed any change in the vaginal smears. It will be

noted that the use of glacial acetic acid greatly increased the yield of estrin over that of sulphuric and hydrochloric acids, but the yield was not great enough to produce estrous smears in rats which received 0.75 cc. of the extract, and estrous smears were not found consistently in rats receiving 1.5 cc.

Extracts made after the urine was acidified with trichloracetic acid, however, contained sufficient estrin to give fairly consistent estrous smears after the injection of 1.5 cc. of the extract, and estrous smears after the injection of 0.75 cc. were not unusual. Eighteen assays were done using this acid for acidification, 108 rats being injected. Estrous smears were found in each of the 36 injected with 3.0 cc. of the extract, in 25 of the 36 which received 1.5 cc., and in 9 of the 36 which received 0.75 cc. No pro-estrous smears were observed.

Thirteen assays were done when tartaric acid was used for acidification, 78 rats being used. This allowed 26 rats in each group. All the rats that were injected with 3.0 cc. and with 1.5 cc. showed estrous smears. Twenty-one of the 26 rats injected with 0.75 cc. showed estrous smears. (Table I.)

TABLE I.

Effect of Acidification of Urine on Number of Estrous Smears after Injection of Extract.

Acids Used	Number of Rats in Assays	Number of rats showing estrous smears after injection of extract: Amounts of injections and their equivalents in rat units		
		3.0 cc.-4.6 r.u.	1.5 cc.-9.3 r.u.	0.75 cc.-18.6 r.u.
Sulphuric	90			
Hydrochloric	102	5		
Glacial acetic	144	41	3	
Trichloracetic	108	36	25	9
Tartaric	78	26	26	21

When the results of the extracts made after acidification of the urine with the various acids are interpreted in terms of the formula explained above, it is found that in the extracts from urine which was acidified with sulphuric or hydrochloric acid, the yield of estrin was something less than 4.6 rat units per liter. Glacial acetic acidification caused a greater yield of estrin than acidification with either sulphuric or hydrochloric acid. The average yield of the extract made with glacial acetic acid is 4.6 rat units per liter. Trichloracetic acidification, however, gave an average yield of 9.3 rat units per liter, while tartaric acidification yielded 18.6 or more rat units per liter (Chart 1).

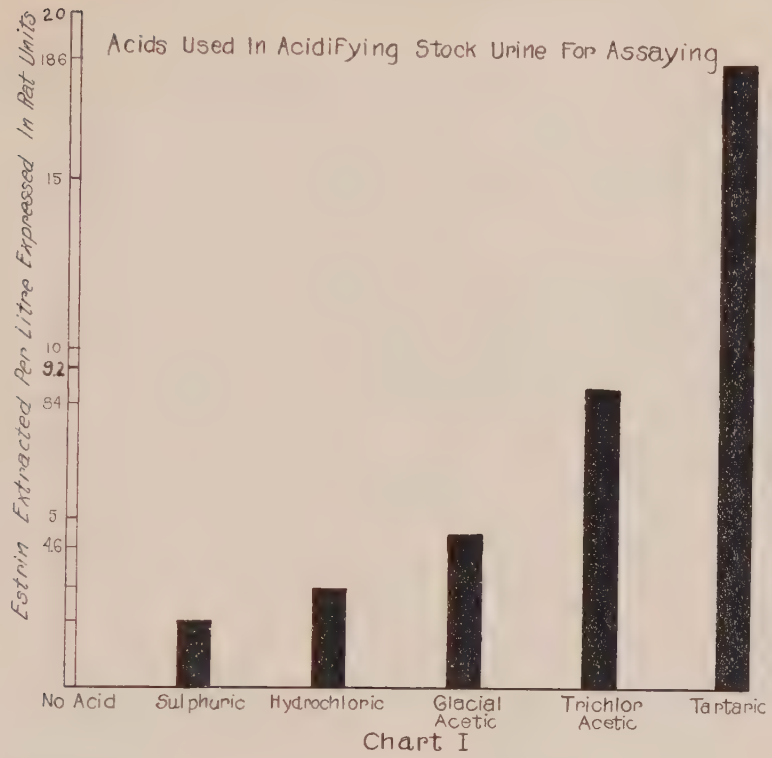

Chart I

Summary. 1. A total number of 87 assays of estrin were done on female stock urine. Fifteen were done after acidification of the urine with sulphuric acid, 17 with hydrochloric acid, 24 with glacial acetic acid, 18 with trichloracetic acid, and 13 with tartaric acid. 2. It has been shown that, by the use of the described method, varying yields of estrin are secured by extraction and assay of urine which has been acidified with various acids. 3. Extractions made after acidification with sulphuric and hydrochloric acids gave negative results. 4. Extracts made after the acidification with glacial acetic, trichloracetic, and tartaric acids all contained measurable amounts of estrin. 5. Of the 3 acids mentioned, urine acidified by the use of tartaric acid produced the greatest yield of estrin as assayed by the method described in this paper.

The author wishes to express his appreciation to Miss Bernice Morrison for her valuable technical assistance.

8093 P

Effect of Epinephrin on Normal and Diabetic Response to Insulin.

B. N. BERG, J. MCAFEE AND T. F. ZUCKER.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

The search for a cause of the marked difference in the course of action of insulin intravenously injected in the normal and depancreatized dog[1] brings up the question whether epinephrin secretion in response to low blood sugar can be responsible for the rapid return to the initial level which sets in at 20 to 25 minutes in the normal animal.

When the action of 3 mg. of epinephrin (3 cc. of 1:1000 solution) is superimposed on insulin action in normal and diabetic dogs they still show respectively a curve with a definite minimum at 20 minutes and a continuous drop to at least 60 minutes. Eight experiments were carried out to cover a reasonable dose range of both drugs. An example is given in Table I.

TABLE I.

Dog No. 211 (Normal) With epinephrin 10 min. before injection of 0.1 u. of insulin per kg.		Dog No. 209 (Depancreatized) With epinephrin 10 min. before injection of 0.1 u. of insulin per kg.	
Min. after insulin	% of initial blood sugar value	Min. after insulin	% of initial blood sugar value
0	100	0	100
10	68	10	102
16	59	20	93
20	64	31	88
24	69	41	85
32	63	51	79
38	73	61	78
42	77	72	84
44	88	81	88
63	94	110	104
78	103	141	135
104	119		

According to the data of Hrubetz[2] the amount of epinephrin given should be more than enough to counteract the insulin effect if the 2 drugs were direct antagonists. Control experiments where epinephrin alone is given, as well as the late blood sugar rise when both drugs are given, indicate also that the epinephrin dose is large

[1] Berg, B. N., Gross, J., McAfee, J., and Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1080.

[2] Hrubetz, M. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 218.

enough to meet the requirements of the experiment, *i. e.*, give a blood sugar raising effect equal to the blood sugar lowering effect of the insulin. Against a background of several hundred blood sugar curves we would judge that with the doses here used the epinephrin has little effect on the course of insulin action and asserts itself only after the action of the latter has passed off.

The lack of rapid return of blood sugar towards the initial level in the diabetic animal cannot be attributed to absence of mobilizable liver glycogen. Epinephrin alone causes the usual mobilization of sugar in the diabetic dog. When both drugs are given the delayed blood sugar rise also testifies to the presence of liver glycogen.

Unless secreted epinephrin has an altogether different action from injected epinephrin the conclusion seems warranted that the normal type of response to intravenous insulin with its early return to the normal is not due to epinephrin secretion.

8094 C

Placental Immunity. 1. A Method of Determining Dosage of Placental Globulin in Measles Prophylaxis.

SAMUEL KARELITZ, CHARLES K. GREENWALD AND A. J. KLEIN.
(Introduced by B. Schick.)

From the Department of Pediatrics, Mt. Sinai Hospital, and Bureau of Laboratories, Department of Health, City of New York.

Globulin extracted from human placenta has been found by McKhann and Chu[1] to be effective in measles prophylaxis. Our experiences with this preparation confirm their results. The dosage employed by us has, however, been larger than those previously recommended, and the percent of our cases completely protected was smaller. One reason for this discrepancy is that we treated only those children who were intimately exposed to measles in their own homes, and did not include children who were presumably exposed in hospitals, nurseries, schools, buses, playgrounds, etc. Results of measles prophylactic studies[2] made under such conditions are more accurate than those of institution epidemics.

The dosage of globulin extract from placenta is being determined by injecting various amounts under comparable conditions of exposure to children of a definite age range, as has previously

1 McKhann, C. F., and Fu Tang Chu, *Am. J. Dis. Child.*, 1933, **45**, 475.
2 Karelitz, S., and Schick, B., *J. Am. Med. Assn.*, 1935, **104**, 991.

been worked out for convalescent serum and immune adult blood. Since placentas vary in size, and in the amount of free blood obtained with them, and probably vary considerably in the amount of immune substance they contain, one can determine dosage in the manner mentioned above only when large numbers of placentas are pooled.

The determination of the prophylactic value of placental extract on the basis of its nitrogen content does not seem to be sufficiently reliable as yet, if we apply the knowledge that 2 samples of diphtheria antitoxin with the same nitrogen content may differ widely in their antitoxic titres. Our data indicate that this is also true of the placental globulin extracts. (Table I.)

TABLE I.

	—Globulin Extract—		Diphtheria Antitoxic Titre			Measles Prophylaxis Dose B/A times 10
	Quantity	Nitrogen	A—Extract	B—Mat. Serum	Nitrogen	
Placenta	cc.	mg. %	units/cc.	units/cc.	mg. %	cc.
1	100		.05	.05	2.48	10
2	"		.06	.09	2.48	15
3	"		.03	.04	2.44	13.3
4	"	3.72	.01	.02	1.92	20
5	"	2.67	.03	.03	1.37	10
6	"		.19	.11	1.55	5.7
7	"		.03	.02	1.83	6.6
8	"	2.56	.06	.05	1.98	8.3
9	"	3.75	.33	.11	2.28	3.3
10	"		.15	.06	2.18	4

Dosage of Globulin Extract is calculated to compare with 10 cc. of immune adult blood serum: If the comparison were to be made with a different quantity (X) of blood serum the calculation would be B/A times X, instead of B/A times 10, as above.

Both for academic as well as practical reasons it would seem desirable to be able to determine in each placenta the prophylactic value of its globulin content. To this end we (Karelitz) have suggested a working hypothesis—a procedure based on the following facts: 1. McKhann and coworkers[3] have found that the placental globulin extract as prepared by them contains both the diphtheria antitoxin and the measles antisubstance, when present at all. We[4] have confirmed these findings. 2. It has been shown[5] clinically that the globulin extracted from measles immune human blood serum is equally effective in measles prophylaxis as the equivalent quantities

[3] McKhann, C. F., and Coady, Harriet, *Southern Med. J.*, 1934, **27**, 20.

[4] Unpublished data of authors.

[5] Karelitz, S., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 793.

of blood serum. 3. The dosage of immune human blood serum in measles prophylaxis has been established in previous studies, to vary between 8-40 cc.

The deduction we have drawn is that the measles prophylactic value of that amount of human blood serum which contains one unit of diphtheria antitoxin should be equivalent to the amount of placental globulin extract which contains one unit of diphtheria antitoxin. For example: Blood serum obtained from a woman at the time she expels the placenta contains 0.05 units of diphtheria antitoxin per cc. The globulin extracted from her placenta has a diphtheria antitoxic titre of 0.10 units per cc. The placental globulin therefore contains twice the quantity of diphtheria antitoxin per cc. as does the circulating blood serum of this same person. Since it is known that the dosage of adult blood serum will vary from 8-40 cc., we believe, if our hypothesis is correct, that in this particular instance we would use just half as much globulin extract, namely 4-20 cc.

We have attempted to test our hypothesis in the following experiment: Blood was taken from 20 women at the time of the expulsion of the placenta. The bloods were permitted to clot and the diphtheria antitoxic titres of their sera were measured by the neutralization test. The placentas were individually extracted as has been suggested in the original paper by McKhann and Chu, and these globulin extracts, after being each diluted to 100 cc. volume, were studied for their diphtheria antitoxic titre. In the manner illustrated by the example cited the dosage of the various globulin extracts was determined, using the results obtained with 10 cc. of immune adult blood serum as the basis for our comparison.

Using the method described for arriving at the dosage of placental globulin extract, we have injected 60 children with the preparation from 10 placentas. As will be noted the dosage of globulin extract varied from 3.7 cc. to 20 cc., or from 5-30 doses from a placenta.

Our results are very similar to those obtained by various investigators[6, 7] with dosage of 10 cc. of adult serum. (Table II.)

The results indicate that our hypothesis was correct, since the placental extract and 10 cc. of adult serum yielded similar results. As pointed out above we calculated the dosage of globulin extract to be compared with 10 cc. of adult serum. The individual extracts

[6] Morales, E. G., and Mandry, O. C., *Am. J. Dis. Child.*, 1930, **39**, 1214.

[7] Karelitz, S., and Schick, B., *J. Am. Med. Assn.*, 1935, **104**, 991.

TABLE II.

	Dosage	No. Cases	Protected %	Modified %	Failed %
Morales & Mandry	10 cc. A.S.	138	40	40	20
Karelitz & Schick	10 cc. A.S.	70	42	41	17
Authors	3.3-20 cc. Globulin Extract	60	37	43	20

A.S.—Adult Serum.

are at present difficult to compare because of the small numbers of case reports received to date.

In the event that there is no diphtheria antitoxin either in the placenta or maternal circulation, this method fails. If, however, several placentas are pooled, it is possible to compare the diphtheria antitoxin of the globulin extract of the pooled placentas with the diphtheria antitoxic titre of the pooled specimen of 1 cc. of blood serum obtained from each mother at the time of expulsion of the placenta.

Conclusions. 1. By comparing the titre of diphtheria antitoxin of maternal blood and the globulin extracted from a placenta one may arrive at equivalents which are also equivalent in measles prophylaxis. This offers a method of measuring dosage of placental globulin extract. Ten experiments performed indicate that this, with the exception cited, is true. 2. The measles antibody and diphtheria antitoxin are both contained in the globulin fraction as extracted. 3. Globulin extract when properly applied as to dosage and conditions of exposure is effective in measles prophylaxis.

8095 C

Placental Immunity. 2. Comparison of Maternal Circulating Blood Immunity with that of Placental Fluid.

SAMUEL KARELITZ AND CHARLES K. GREENWALD. (Introduced by B. Schick.)

From the Department of Pediatrics, Mt. Sinai Hospital, and Bureau of Laboratories, Department of Health, City of New York.

In their study of the prophylactic value in measles of the globulin extracted from the human placenta McKhann and Chu[1] raised the question of the source of these antibodies. They believed that besides maternal and fetal blood there were possibly antibodies originating in the placental tissue as such.

[1] McKhann, C. F., and Fu Tang Chu, *J. Infect. Dis.*, 1933, **52**, 268.

The demonstration by various authors that the titre of cord blood and maternal circulating blood for diphtheria antitoxin, scarlet fever antitoxin, and poliomyelitis virus neutralizing substance are alike in most instances, suggests the direct transplacental transmission of antibodies from the mother to the child.

We[2] reported that the globulin extracted from immune adult males' blood in the same manner as suggested by McKhann for placenta was equally effective in measles prophylaxis as the blood itself. In attempting to explain the striking results of McKhann and coworkers we suggested that possibly all of the extracellular placental fluid may be comparable to blood serum in its antibody content. In the course of several studies on placental immunity we obtained some evidence which seems to bear out this suggestion.

15-20 cc. of blood was drawn from a superficial vein of the mother at the time of expulsion of the placenta. This blood was permitted to clot, and the diphtheria antitoxic titre of the serum was measured by the toxic neutralizing test. The placenta plus what blood and fluid dropped into the receptacle with it was collected under sterile conditions. The fluid was decanted. The placenta was cut up into many small sections and squeezed in a simple orange hand press.

The fluid so obtained plus the blood which was decanted were mixed and measured, then centrifuged for 15 minutes under high speed. The sanguinous supernatant fluid was measured and its diphtheria antitoxic titre determined.

TABLE I.

	Placenta				Diphtheria Antitoxic Titre	
Nos.	Wt. Gm.	Fluid A	Supernatant Fluid B	B/A %	Placental Fluid U/cc.	Maternal Blood Serum U/cc.
1	880	150			.05	.05
2	755	150			.03	.03
3	1340	175			.05	.05
4	1050	360	290	80	.20	.20
5	900	90			.06	.06
6	575	33			.14	.16
7	580	140	110	78	.00	.00
8	770	225	155	69	.00	.00
9	585	125	75	60	.00	.00
10	920	325	210	65	.00	.02

As seen in Table I, in 8 out of 10 instances the antitoxic titre of the maternal circulating blood serum and that of the placental fluid were equal, and in the other 2 there was only a slight difference.

[2] Karelitz, S., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 793

The placental fluid obtained as it was, must have been a mixture of maternal and fetal blood, interstitial fluid, some placental tissue, and possibly a slight amount of amniotic fluid. That it was not blood alone may be deducted from the fact that after centrifuging, the supernatant fluid varied from 60-80% of the total, instead of 40-45% as is usually obtained when blood is centrifuged. In addition the residue contained some placental fibres in all instances. It is not likely that laking of some of the blood caused this high percent of supernatant fluid.

Since the antitoxic titre of this supernatant fluid was identical with maternal blood serum, we may deduce that the interstitial fluid and placental blood probably have the same diphtheria antitoxic titre as does the maternal blood serum.

This evidence added to the fact that cord blood contains the same diphtheria antitoxic titre as does maternal blood, indicates that the immune substances of the placenta are probably due entirely to *passive immunity* transmitted from the mother to the placenta and from there to the fetus. Active immunity would result in such quantitative agreement between blood and placental fluid with such regularity, only if there is a constant interchange of antibodies between mother, placenta and fetus.

The determination of the diphtheria antitoxic titre of amniotic fluid is now in progress. In the first case analyzed no antitoxin was found in the amniotic fluid and none (less than 1/100 unit per cc.) was found either in the placenta or in the maternal circulating blood.

The information obtained in this study suggested its practical application in measles prophylaxis.

It has been shown by McKhann and Chu[3] and confirmed by us that the globulin, as extracted, contains the measles antibody as well as the diphtheria antitoxin. If the measles antibody is present at all, it may be assumed from the evidence presented that it would be contained in this placental fluid in the same proportion as in the serum of the maternal circulation, since that was true of the diphtheria antitoxin.

We might therefore use similar quantities of this fluid as we would use immune adult blood serum in measles prophylaxis. The truth of this hypothesis is now being tested.

If the placental globulin extract is used, its dosage may be determined by comparing the antitoxic titre of the extract with that of the placental fluid, instead of maternal blood as was suggested in a

[3] McKhann, C. F., and Coady, Harriet, *Southern Med. J.*, 1934, **27**, 20.

previous paper.[4] This may be done because of the similar diphtheria antitoxic titre in maternal blood and placental fluid.

Conclusions. 1. The fluid obtained by squeezing the human placenta has the same diphtheria antitoxic titre as does the serum of the circulating blood of the mother drawn at the time of expulsion of the placenta. 2. This fluid is a mixture of maternal and fetal blood and tissue fluid, and possibly also a slight amount of amniotic fluid. 3. The diphtheria antitoxin titre of the placental blood, the circulating blood of the mother, and the placental tissue fluid are alike in most cases. 4. The antibody content of the placenta is probably entirely the result of transplacental transmission of the immune substance from mother to placenta, to fetus, and is probably passive in nature. 5. Since the globulin fraction carries with it both the diphtheria antitoxin and the measles antibody, it is suggested that this fluid be used in measles prophylaxis. 6. If the globulin of pooled placentas is to be used in measles prophylaxis, its dosage may be calculated by comparing its diphtheria antitoxic titre with that of the pooled placental fluid, and thus the necessity of obtaining maternal blood is obviated.

8096 C

Effects of Heavy Water on Mammalian Metabolism.

HENRY G. BARBOUR.

From the Department of Pharmacology and Toxicology, Yale University.

Heavy water has hitherto not been subjected to serious pharmacological study in mammals; indeed it has been uncertain whether such activity exists to any significant extent. From its unusual physical characteristics, however, and its effects upon the behavior of lower forms of life (Urey,[1, 2] Barnes[3]) depression of function has been generally predicted in mammals. In particular, Barnes[4] has observed, for example, decrease in the rate of activity of the con-

[4] Karelitz, S., Greenwald, C. K., and Klein, A. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1359.

[1] Urey, Harold C., Cold Spring Harbor Symposia on Quantitative Biology, 1934, **2**, 47.

[2] Urey, H. C., and Teal, G. K., *Rev. Mod. Physics*, 1935, **7**, 34.

[3] Barnes, T. Cunliffe, and Jahn, Theo. L., *Quart. Rev. Biol.*, 1934, **9**, 292.

[4] Barnes, T. Cunliffe, and Gaw, H. Z., *J. Am. Chem. Soc.*, 1935, **57**, 590.

tractile vacuoles of protozoa and finds in 30% heavy water a reduced contraction rate supporting the prediction of chemists that deuterium will be found to have effects similar to those of low temperature.

The oxygen consumption of luminous bacteria in high concentrations of heavy water has been found decreased by Harvey and Taylor,[5] using the Warburg method. The same is true of yeast in 20% (or higher) D_2O. Other instances of depressed function might be cited; that reported in the highest form of life thus far is apparently the slowing of the frog heart recently described by Barnes.[6]

Our starting point in mammals has been a study of the metabolic rate of 2 white mice, each treated with several subcutaneous injections of 99% deuterium oxide. The carbon dioxide and water output, oxygen consumption and respiratory quotient have been closely followed, parallel with the variation in apparent content of the body in heavy water.

The method employed was a Haldane open metabolism chain which included some new features which will soon be published in detail by Barbour and Cochran.[7] An essential feature consists in the recovery of 97.5% of the insensibly lost water by passing it through a 100 cc. pipette, surrounded by well-insulated dry ice. Caught in this fashion, the insensibly lost water of the mouse assumes in 24 hours the appearance of a volume of snow nearly as large as the animal itself. When melted 0.02 cc. of the material suffices for a duplicate determination of the specific gravity by the falling drop method. The balance of the material may be again injected under conditions requiring low concentrations of D_2O.

The metabolic determinations were made in runs lasting from 9 to 12 hours.

The 2 mice used, No. 12 and No. 16, respectively weighed 20 and 23.8 gm. For Mouse No. 12, the routine procedure was to make 2 runs in 24 hours, separated by periods of ½ to 1 hour, in which food was allowed. Just before each run, *i. e.*, twice daily, 1.0 cc. of water was injected subcutaneously. No other water was allowed and the food uniformly employed ("Fox Chow") was practically dry. After 6 preliminary runs, Mouse No. 12 was injected with 1 cc. of 99% D_2O instead of H_2O before each of the next 4 runs (see

[5] Harvey, E. Newton, and Taylor, G. W., *J. Cell. Comp. Physiol.*, 1934, **4**, 357; PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 954.

[6] Barnes, T. C., and Warren, J., *Science*, 1935, **81**, 346.

[7] Barbour, H. G., and Cochran, F., *Science*, 1935, in press.

dark block in Fig. 1). No H_2O at all was allowed during this period. The effect was so immediate that the first run showed a depression of the metabolism to about one-half of the average normal figure, a level which was maintained for the next 2 runs; the fourth and final dose of heavy water reduced the metabolism still lower. These effects are seen in the CO_2 curve and the somewhat similar water loss curve. Using for the surface area of the mouse, Benedict's[8] modification of Rubner's formula: $9.W^{2/3}$ and calculating the calories from the oxygen determinations, we find that the metabolism of this mouse fell from an average normal of 1,925 cal./sq.m./24 hr. to a minimum of 751 cal./sq.m./24 hr. Eight days after resumption of the H_2O injections, the metabolism had begun to return toward normal, but following this run the mouse was accidentally asphyxiated.

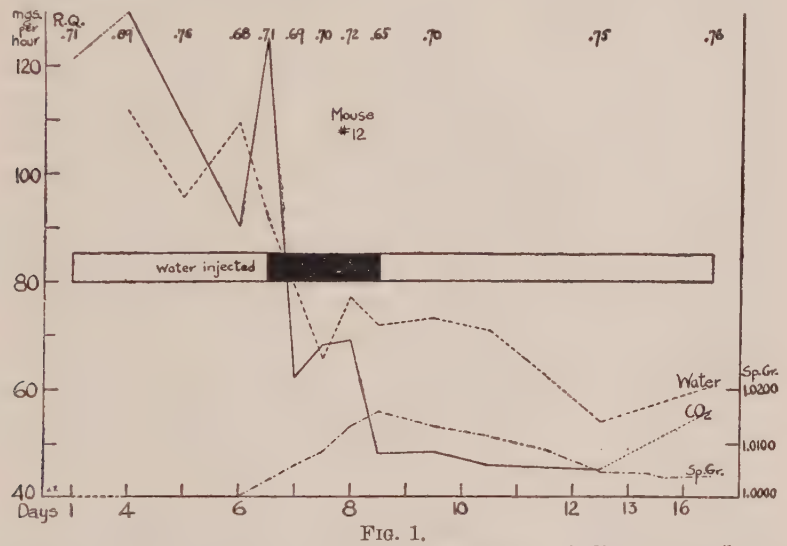

FIG. 1.

Mouse 12. Effects of heavy water on metabolism. Ordinates, mg./hr.; abscissae, days; heavy lines, CO_2 output; upper broken lines, water output; lower broken lines, specific gravity of insensibly lost water. One cc. H_2O injected twice daily during periods indicated by light blocks, D_2O dark block. Top line: R.Q. (in figures).

Finding that mouse No. 12 had received hardly enough water to meet the requirements of his combined output by urine and evaporation and desiring also that food be made accessible for a longer period each day, it was decided to provide the second mouse, No. 16,

[8] Benedict, Francis G., and Fox, Edward L., *Pflüger's Arch. f. d. ges. Physiol.*, 1932, **231**, 455.

with an over-abundance of water by injection and to make food available for at least 12 hours daily. To this end the mouse was left each day from about 7 a. m. until 7 p. m. in a beaker with abundant food and the metabolism runs were made only during the night period. After a study of the normal mouse for 6 days in this fashion, 3 successive night runs were made before each of which was given a large dose (1.5 cc.) of heavy water (see black blocks in Fig. 2). After each night's run the injection consisted chiefly of H_2O, but with the addition of sufficient D_2O to maintain approximately the same concentration of the latter in the body, as shown by the specific gravity of the insensibly lost water.

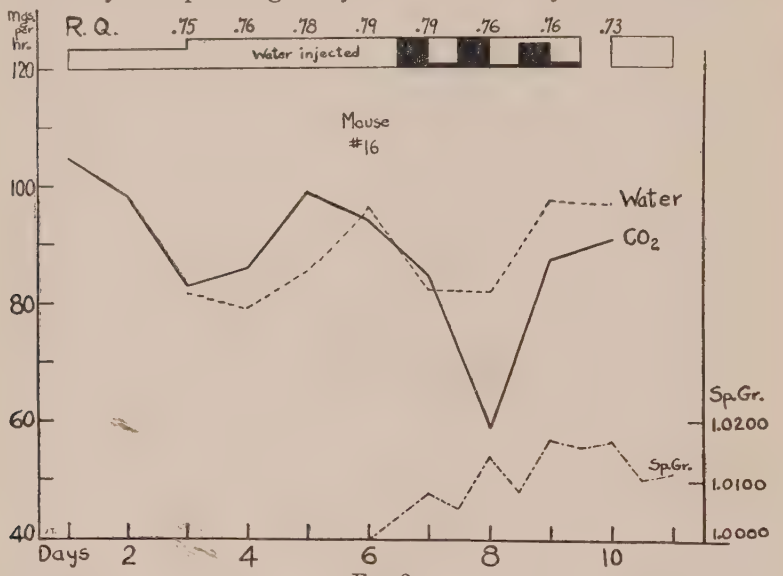

FIG. 2.

Mouse 16. Same as Fig. 1, except dose of water injected raised on third day to 1.5 cc. injected twice daily. Areas of mixed light and dark indicate proportional amounts of H_2O and D_2O injected together.

In this well-fed and well-watered mouse the depression of metabolism was much less striking. There was, however, a reduction from the normal average of 1304 cal./sq.m./24 hr. to a minimum of 868 cal./sq.m./24 hr., reached after the second night's run. Before the third night's run it was necessary (to make up the 1.5 cc. dose) to add H_2O to the D_2O injected; the metabolism of this run returned to nearly the normal level.

The specific gravity of the insensibly lost water was determined after each run and in each case a figure of over 1.0150 was reached

after the last heavy water injection. Since the specific gravity of pure deuterium oxide is 11.079, this figure is interpreted as suggesting that at this point each mouse was about 13.5% saturated with heavy water. The exact equivalence, however, of the D_2O/H_2O ratio in insensibly lost water to the ratio existing in the body remains still to be quantitatively determined.* Inasmuch as the second mouse showed a slower and less decisive fall of the metabolism, it is argued that a better condition of hydration with H_2O at the onset of a heavy water experiment tends to protect against the effect of the latter substance. This hypothesis that more subtle penetration of D_2O can be obtained in dehydrated animals remains to be tested.

So far as can be judged, both mice showed as great activity throughout the heavy water treatment as in their previous condition. The only exception to this was a slight depression in the respiratory rate observed in each mouse after the first D_2O run. They appeared otherwise to be as alert and to eat as much at all times. There is no reason to ascribe the fall in metabolism to diminution either in activity or in food intake. This statement is well borne out by the maintenance throughout the D_2O periods of essentially the same respiratory quotient as found during the immediately preceding normal periods. The question of true basal metabolism in the mouse has been dealt with by Benedict and Fox[8]; while the present work does not probably present the mice at their basal levels of metabolism, there is no reason to doubt that the chief factor in the metabolic fall seen in each case was the inhibiting effect of deuterium oxide upon the activity of the cells.

8097 C

Effects of Light and Dark Environment on Weight Changes in Normal and Hypophysectomized Frogs.

MILDRED E. JONES AND F. R. STEGGERDA.

From the Department of Physiology, University of Illinois, Urbana.

It is a well established fact that the pituitary gland of the frog plays an important rôle in the dilatation and constriction of the melanophores in dark and light environments. It was shown by

* It may be disturbed by the loss of deuterium to carbohydrate and urea.

one of us[1,2] that injections of extracts of the posterior lobe of the pituitary gland produce a marked increase in water uptake in frogs as well as dilatation of the melanophores. Subsequently we became interested in the problem as to whether or not environmental background had any effect on the rate of weight changes in frogs kept constantly in water. The solution of this problem we feel, would help to determine whether the weight increase in frogs, resulting from the injection of pituitrin, is actually related to dilatation of the melanophores or to a more specific skin permeability.

Two sets of experiments were carried out. In the first, 3 different groups each containing 6 normal frogs, were removed from water, dried carefully with gauze, and then weighed accurately to 0.1 gm. They were then put back in water on a white background. Within 12 hours they had responded to this environmental change by becoming lighter in color, due to a contraction of melanophores. After 4 days in this environment they were again removed, dried, weighed, and then transferred to a black background to which they responded by a dilatation of melanophores, and where they remained for 4 more days. Following this they were transferred to a neutral background which consisted of a galvanized tank, for the same interval of time, and they were finally transferred back to the white background for the last 4 days of the experiment. Thus, each group of frogs was exposed to 4 different environmental backgrounds in 16 days with weights recorded between each change.

In the second set of experiments 6 frogs, hypophysectomized 4-5 days previous to the experiment to preclude melanophore changes, were exposed to changes in environmental background and weighed in the same way as were the normal frogs for the 16-day period.

The results show that all of the frogs in both the control groups and the hypophysectomized group, lost weight continuously from the beginning of the experiment until the end regardless of the change in environmental background. The final weight loss for each group after a period of 16 days was as follows: 12.5%, 8.8% and 7.7% for the control groups, and 12.3% for the hypophysectomized group. The individual variations in weight loss amounted to $\pm 3\%$ for the control group and $\pm 7\%$ in the experimental group. It was reported by Dietel[3] that in his experiments hypophysectomized frogs gained in weight, but usually died after 4 or 5 days. In

[1] Steggerda, F. R., *Am. J. Physiol.*, 1931, **98**, 255.

[2] Steggerda, F. R., and Essex, H. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 425.

[3] Dietel, F. G., *Klin. Wochen.*, 1932, **11**, 2075.

the present work, such results were obtained only in cases where the brain had been injured in the process of removing the pituitary.

The experiments were done during the months of February and March on different shipments of frogs at room temperatures estimated to be about 22°C. with a fluctuation of not more than ±3°C. These factors may be partly responsible for individual variations in weight losses in the different groups, although it is admitted that the nature of the operation may be responsible for the wider variation in the hypophysectomized group. Since there is no real consistent difference in rate of weight loss in frogs with or without the power (due to hypophysectomy) of dilatation and constriction of melanophores, we conclude that changes of background have no effect on the ability of the frog to retain or lose water.

8098 P

Contracture of the Rectus Abdominis, Tetany, Calcium and Phosphorus, after Spinal Transection, with and without Thyroparathyroidectomy.

HELEN C. COOMBS, JOHN ABAJIAN AND F. H. PIKE.

From the Department of Physiology and Physiological Chemistry, New York Homeopathic Medical College, and Columbia University.

As one phase of some observations on the effects of parathyroidectomy on cats,[1] a previous study[2] showed that on stimulation of the rectus abdominis muscle following thyroparathyroidectomy, more contracture usually appeared than was the case with the same muscle stimulated in the same manner in control animals. During the past months a series of experiments has been done on 20 cats in which the ionizable calcium and inorganic phosphorus of the blood were determined from 2 to 3 days after (1) aseptic transection of the spinal cord alone in the upper thoracic region and (2) when thyroparathyroidectomy was done at the same time as the spinal transection. The working power of the curarized muscle, together with the presence or absence of tetany and of contracture, was determined in the same manner as in previous experiments.

The results are given in Table I .

[1] Coombs, H. C., Searle, D. S., and Pike, F. H., *Am. J. Psychiatry*, 1934, **13**, 761.

[2] Coombs, H. C., and Searle, D. S., *Am. J. Physiol.*, 1934, **109**, 23.

TABLE I.

Section of Spinal Cord						Section of Spinal Cord and Thyroparathyroidectomy					
No.	Ca mg./100cc.	P mg./100cc.	Ca/P	Contr.	Tetany	No.	Ca	P	Ca/P	Contr.	Tetany
4	10.1	6.9	1.4	+	—	2	7.2	8.2	.88	—	+
6	8.1	15.3	0.5	+	—	3	6.5	8.8	.73	—	+
8	10.6	11.0	0.9	—	+	5	4.6	7.1	.64	—	+
11	10.1	6.0	1.7	+	+	7	9.0	8.3	1.0	—	+
12	10.4	5.9	1.7	+	+	9	6.3	7.1	0.9	+	+
15	11.1	6.1	1.8	—	—	10	10.4	4.0	2.5	+	+
17	11.1	8.3	1.3	+	—	13	4.8	8.9	.54	—	+
19	10.3	6.1	1.7	+	—	14	7.5	8.4	.90	—	+
21	11.1	6.1	1.7	+	—	16	9.0	5.9	1.4	+	+
						18	7.1	6.0	1.2	+	+
						20	8.0	5.9	1.3	+	—

It appears from the table that 1. Either contracture or tetany was present in all but one case, in which the contracture occurred at the beginning of the excitation of the muscle and was merely transitory. 2. Contracture and tetany were found together in only 6 of the 20 experiments. There appears to be no necessary relationship between them. 3. Contracture did not occur when the ratio of calcium to phosphorus was less than one, except in one case (No. 9). From these and previous observations it would appear that contracture occurs infrequently when the ratio of calcium to phosphorus is less than one, and then only when the calcium is relatively low; on the other hand, contracture was observed in only one case where the concentration of phosphorus was above 8 mg. per 100 cc. serum. (No. 6.) In those experiments in which total protein was estimated (No. 14-21), the range was from 5.9 to 7.3 gm. Values within this range are not considered pathological.[3]

8099 C

Course of Phosphatase Activity in Healing of Fractured Bone.

WALTER E. WILKINS AND EUGENE M. REGEN. (Introduced by C. S. Robinson.)

From the Departments of Biochemistry and Surgery, Vanderbilt University School of Medicine, Nashville.

Robison[1, 2, 3] suggested that the enzyme phosphatase may play an important part in the deposition of phosphates in growing bone. Later studies by Robison and his coworkers, Kay[4] and other authors have furnished much additional evidence in support of this concept. Most of this work has dealt with the growth of bone, and the study of this enzyme in relation to regeneration of bone has been largely neglected. Studies by McKeown and Ostergren[5] and Kamada[6]

[3] McLean and Hastings, *J. Biol. Chem.*, 1935, **103**, 295.

[1] Robison, R., *Biochem. J.*, 1935, **17**, 286.

[2] Robison, R., The Significance of Phosphoric Esters in Metabolism, New York, 1932.

[3] Robison, R., *Ergebnisse der Enzymforschung*, 1932, **1**, 280.

[4] Kay, H. D., *Physiol. Rev.*, 1932, **12**, 384.

[5] McKeown, R. M., and Ostergren, J. I., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 54.

[6] Kamada, K., *Fukuoka Acta Med.*, 1932, **25**, 207. Quoted from *Chem. Abst.*, 1932, **26**, 6002.

indicate that phosphatase may play a part in the healing of fractured bone.

The object of the present study was to determine the course of the phosphatase activity of fractured bone throughout the period of healing. The general plan of the experiment was to produce fractures in a large series of uniform animals, to sacrifice them in groups, at different stages throughout the period of repair, and to analyze the bone at the site of fracture for its phosphatase activity.

The estimation of the activity of the enzyme at the site of fracture necessitates the removal of the bone, and in order to study the phosphatase activity at different stages it was necessary to use a series of animals. In order to avoid the influence of growth on the enzyme, old animals were used. This group consisted of 20 female, pedigreed, New Zealand white rabbits, each approximately 2 years of age. We feel that these animals are as nearly identical for experimental purposes as can be obtained. Throughout the experiment the animals were kept at the registered rabbitry from which they were bought.

Under nembutal anesthesia, by open operation, and with aseptic precautions, fractures were produced in both ulnae by cutting with bone forceps near the middle of the shaft. This resulted in extensive comminuted fractures in each case. The wounds were closed with silk and sealed with collodion. Fractures were produced in this manner in 14 animals. No wound infection occurred. Six animals were used to determine the level of the phosphatase activity in normal unfractured foreleg bones.

It had been learned from a few preliminary experiments which had been previously carried out on a small series of similar animals that after fracture there is a rapid rise followed by a prolonged decline in the phosphatase activity at the site of injury. On the basis of these findings the animals were sacrificed in groups of two, 1, 3, 10, 22, 40, 56, and 89 days after fracture as shown in Fig. 1. The bones were removed and thoroughly cleaned, care being taken not to displace any of the soft granulations from those bones which were used during the early stages of healing. The radius and ulna were cleaned without being separated and a section of both of these bones at the site of fracture was removed with a motor saw. Each section was cut as short as possible to include all the fracture. That portion of the radius adjacent to the fracture was included since its removal during the early stages of healing would cause movement at the fracture and loss of tissue fluid. Thus the fracture was left intact until the sample had been weighed and prepared for extraction.

The 2 samples from each animal were pooled for analysis. Similar sections were taken from normal unfractured shaft bones of 2 of the control animals. Phosphatase determinations were made as described in a previous study.[7]

The results of these analyses and the gross condition of the fractures at the time of removal are shown in Fig. 1. Each column

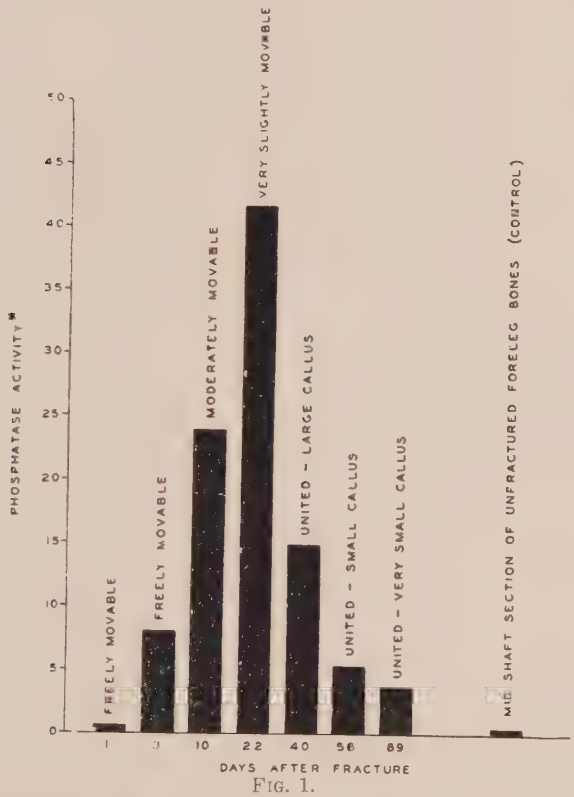

FIG. 1.

Course of Phosphatase Activity in Healing of Fractured Adult Rabbit Bone. Each column represents average value of 4 fractures (2 from each of 2 animals), sacrificed on the days indicated. Condition of fractures in respect to mobility is indicated above each column. Last column represents average activity of unfractured shaft bone of the control animals.

[7] Wilkins, W. E., and Regen, E. M., *Radiology*, 1934, **22**, 674.

* The phosphatase activity is expressed as the number of milligrams of inorganic phosphorus liberated per hour by the enzyme contained in the entire water extract of the sample from a substrate of sodium-Beta-glycerol-phosphate buffered with glycine-NaOH, at a pH of 8.8 and a temperature of 38°C. The activity of the enzyme is calculated on the basis of the entire water extract rather than per unit of volume, since this is a better index of the "amount" of phosphatase present in the healing tissues.

represents the average value of 4 fractures (2 from each of 2 animals). One day after fracture the phosphatase activity was still within the range of that of normal unfractured shaft bone of the control animals. Observation on the third, tenth and twenty-second days showed a rapid rise in the activity of the enzyme. Since only one determination was made between the tenth and fortieth days, it is obvious that the value obtained on the twenty-second day does not necessarily represent the highest point reached. Observations on the fortieth and fifty-sixth days showed a marked drop in phosphatase activity. But even after 89 days it had not returned to the normal level. It is likely that the true peak occurred near the twenty-second day since values obtained 12 days before and 18 days after the twenty-second day are considerably lower. In any case the period of decline was much more prolonged than the period of increase.

Plasma phosphatase determinations were made on some of the animals at the time they were sacrificed. During that part of the experiment when the phosphatase activity at the fracture site was highest, there was an increase in the plasma phosphatase by comparison with the controls. However, in both groups the activity was quite low and the significance of the changes is questionable.

It was not planned originally to study the possible generalized effect of fracture on uninjured portions of the skeleton, but in 2 of the experimental animals, those sacrificed on the fifty-sixth day, phosphatase determinations were made on the remaining portions of the foreleg bones, after the section including the fracture had been removed. In each case it was within the range of normal unfractured whole foreleg bone. This suggests that the increase in the enzyme activity is more or less limited to the site of injury. Further studies on this problem are in progress.

Summary. Following fracture of adult rabbit bone there is a rapid rise in the phosphatase activity at the site of bone injury which reaches a peak at some time near the twenty-second day and drops gradually as repair progresses.

8100 C

Effect of Oestrin on Ovaries and Adrenals.

H. SELYE, J. B. COLLIP AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal, Canada.

The enlargement of the ovary observed during gestation in the rat is solely due to the corpora lutea attaining a much larger size than those of the normal cycle or those produced in non-pregnant animals by administration of gonadotropic substances; yet there has been little investigation of the factors regulating the size of the individual corpus luteum. We have shown[1] that the mere distension of the uterine cavity with paraffin leads to prolonged vaginal dioestrus and inhibits the involution of the corpora lutea of pregnancy even though the embryos and their placentae be removed; these corpora, however, showed histological signs of fatty degeneration and were unable to maintain the mammary glands in a fully developed condition. The hypophysis is not essential for the maintenance of the corpora lutea of gestation.[2, 3] The administration of gonadotropic hormones produces larger corpora in the pregnant animal than in the non-pregnant.[4] Since the gonadotropic hormones lead not only to formation of new follicles and corpora lutea but also to involution of corpora already present, we have repeated and modified these experiments. Six rats received 3 injections of 50 units of A.P.L. (anterior-pituitary-like hormone of pregnancy urine) on 3 successive days at about mid-pregnancy. The animals were sacrificed 7 days later; their ovaries were found to contain very large numbers of corpora lutea, all of which were as large as normal corpora of gestation. There is, therefore, some factor in the pregnant animal which transforms experimentally-produced corpora lutea into corpora lutea of gestation, and maintains them throughout the whole period of pregnancy.

There is evidence that oestrin is produced in large quantities in the pregnant rodent.[5] Wolfe[6] has shown that corpora lutea as

1 Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 488.

2 Selye, H., Collip, J. B., and Thomson, D. L., *Anat. Rec.*, 1934, **58**, 139; PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 589.

3 Pencharz, R. I., and Long, J. A., *Am. J. Anat.*, 1933, **53**, 117.

4 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 530.

5 Selye, H., Harlow, C. M., and McKeown, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**.

6 Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 757, 1192.

large as those of gestation are formed in non-pregnant animals treated with oestrin in large doses. We have confirmed these experiments, and find, in agreement with Scaglione[7] and Hohlweg,[8] that ovaries containing large numbers of large corpora lutea are produced by such treatment. Such observations suggest that oestrin may be the essential factor in the production of the ovarian structure typical of pregnancy; yet the ovaries obtained in these experiments differed from those of pregnancy in that several sets of corpora were present. Attempts were therefore made to study the effects of oestrin in conditions during which follicle maturation is known to be inhibited. In 8 late pregnant rats treated with 500γ of oestrone* in oil daily, pregnancy was prolonged up to 24-26 days, at the end of which time most of the embryos died *in utero,* and only in 2 cases did delivery of the dead fetuses take place. In one case the corpora lutea were still fully developed and apparently functional, judged by their histological appearance and the condition of the endometrium on the 26th day of gestation. It seems that oestrin in these cases prolongs the life of the corpora lutea and thus interferes with parturition. In another experiment, 8 rats received 500γ of oestrone daily in oil from the 4th to the 14th day of lactation. The vaginal smear remained dioestrous throughout; at autopsy on the 10th day of treatment the ovaries, pituitaries and adrenals were found to be greatly enlarged. The average weight of the ovaries was 93 mg., of the adrenals 71 mg., and of the pituitary 18.5 mg. The enlargement of the ovaries in this experiment was due solely to the increase in size of the corpora lutea; the ovary represented in Fig. 1 was the largest of the series, weighing 134 mg., yet the number of corpora lutea was not increased; the stroma of the ovary and those corpora lutea which apparently formed from subsequent ovulations were very poorly developed. The vaginal epithelium of these animals displayed a high degree of mucification, a condition regularly seen in pregnancy but not in lactation, although active corpora lutea are known to be present in both of these states.

We conclude from these experiments that the differences observed when the ovaries, endometrium, and vaginal epithelium of normal pregnant and normal lactating rodents are compared are attributable to the presence of oestrin during pregnancy and its absence during lactation, and that the characteristic picture of the ovary during

[7] Scaglione, S., *Riv. Ital. Ginecol.*, 1930, **11**, 463.

[8] Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.

* The authors are greatly indebted to Professor Girard of the University of Paris for supplying them with large quantities of oestrone used in these experiments.

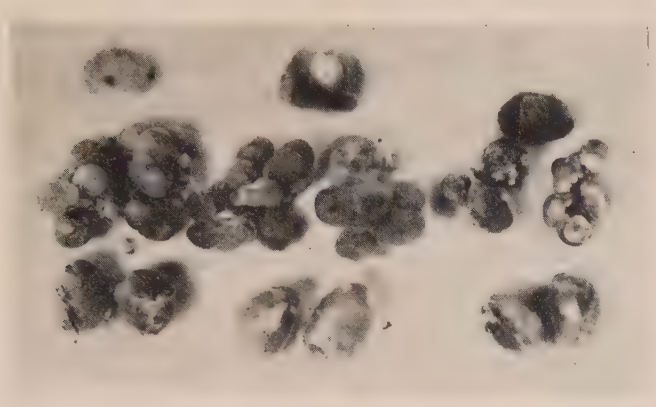

FIG. 1.
Showing that the pituitary, the corpora lutea and the adrenals of the oestrone-treated, lactating rat (in center) are not only larger than those of an untreated control at a corresponding stage of lactation (on the right) but exceed the size of those of a pregnant animal near term (on the left)—the number of the corpora lutea is not increased.

pregnancy is due to the action of oestrin in the presence of some factor inhibiting follicle maturation.

The fact that these observations are in apparent contradiction with those of other investigators[9, 10] who found a decrease in ovarian size after oestrin administration may probably be explained by a difference in the dosage used. In 6 normal adult females treated with 20γ of oestrin daily, the vaginal oestrus was almost continuous and the ovaries showed marked signs of atrophy at the end of 20 days; their average weight was 25 mg., and only in one case did they contain recent corpora. Hohlweg[8] has stated that even large doses of oestrin do not lead to the appearance of corpora lutea in animals much below the age of spontaneous puberty; Lane,[11] however, found evidence of stimulation of follicular maturation in immature rats treated with small doses of oestrin. We have treated a series of 7 21-day-old female rats with 100γ of oestrone daily, in oil; the animals were killed at intervals up to the 30th day of treatment; their ovaries ranged between 7 and 14 mg. and showed definite histological signs of atrophy; spontaneous puberty did not occur in any one of this series. The pituitaries and adrenals were also within the normal weight-range. It appears that oestrin does not lead to

9 Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 702.

10 Doisy, E. A., Curtis, J. M., and Collier, W. D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 885.

11 Lane, C. E., *Am. J. Physiol.*, 1935, **110**, 681.

enlargement of the pituitary or adrenals save in the presence of corpora lutea, nor is such enlargement obtainable in male animals.

It was of interest to ascertain whether the described actions of large doses of oestrin could be maintained over long periods. A series of 12 adult female rats received 500γ of oestrone in oil daily, and were killed at intervals up to 4 weeks. After an initial appearance of vaginal oestrus, a prolonged dioestrus set in, during which the ovaries were seen to be greatly enlarged; after 3 weeks' treatment, however, the ovaries returned to approximately normal weights and vaginal oestrus reappeared. The size of the pituitaries and adrenals ran roughly parallel to the size of the ovaries.

In 12 lactating rats treated with 100γ of oestrone daily the corpora lutea were not maintained after hypophysectomy performed on the 6th day of treatment, to judge by the sudden involution of the mammary glands and the histological appearance of the corpora; vaginal oestrus, however, did not reappear until 5-7 days after the removal of the pituitary. This, however, cannot be interpreted as a sign of maintained activity of the corpora lutea, on account of the following observation: 6 lactating rats receiving as much as 500γ of oestrone daily were ovariectomized on the 6th day of treatment, but vaginal oestrus did not reappear until 5-6 more days had elapsed; no such inhibition was observed in 6 ovariectomized lactating control animals not subjected to pre-treatment with oestrin. The nature of this inhibition of vaginal oestrus is not easy to explain; it may possibly be due to remnants of corpus luteum hormone formed in large quantities under the stimulus of oestrin before ovariectomy not being rapidly excreted; it is also conceivable that organs which hypertrophied under the influence of corpus luteum hormone (like the mammary glands) may inhibit the vaginal effects of oestrin for some time after the ovaries are removed.

It is to be observed that in these experiments rapid involution of the mammary glands followed hypophysectomy, nor did the continued application of large doses of oestrin lead to repair of this involution; we conclude that the presence of the pituitary is requisite for the action of oestrin upon the mammary glands, although Ruinen[12] claims that oestrin causes development of the mammary glands of hypophysectomized male rats. Hypophysectomy also leads to involution of the adrenal cortex in spite of continued administration of large doses of oestrin; we conclude that the effect of oestrin in producing marked enlargement of the adrenals in the

[12] Ruinen, F. H., *Nederland. Tijdsschr. Geneesk.*, 1933, 610.

presence of corpora lutea is an indirect action mediated by the anterior pituitary. Histological examination of the enlarged adrenals obtained by oestrin administration reveals that only small quantities of lipoids are present, whereas very large amounts of lipoid material appear in the cortex within 48 hours of hypophysectomy in spite of continued oestrin administration; these observations are of interest, in that the relation between lipoid content and functional activity of the adrenal cortex has been regarded by various authors from very divergent points of view.

It has been noted[13, 14] that administration of oestrin enhances the luteinizing action of A.P.L. and its effect on ovarian weight; another type of synergism has been noted by Hisaw;[15] others, however, have found the action of gonadotropic substances to be unaffected[16, 17] or inhibited[18, 19, 20] by oestrin. It is in fact easy to obtain synergistic or antagonistic effects at will. 40-day rats show only 30 mg. ovaries after 4 days' treatment with 100 units of A.P.L. if they have been pre-treated with 100γ oestrone daily from the 21st day of life, while 60 mg. ovaries are found in controls not pre-treated with oestrin. On the other hand, 4 rats which received 100γ of oestrone daily from the 21st day of life, and 50 units of A.P.L. daily from the 26th day, possessed ovaries weighing over 165 mg. at the 40th day of life, while the heaviest ovaries in 4 controls receiving the A.P.L. treatment only weighed 64 mg. In short, animals whose ovaries have been depressed by oestrin treatment do not respond normally to A.P.L. administration, while on the other hand corpora lutea produced by the action of A.P.L. are enlarged by concurrent and subsequent oestrin treatment.

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13 Collip, J. B., *Internat. Clinics*, 1932, **4** (42), 65.

14 Magath, M. A., and Rosenfeld, R. M., *Arch. ges. Physiol.*, 1933, **233**, 311.

15 Hisaw, F. L., Fevold, H. L., Foster, M., and Hellbaum, A. A., *Anat. Rec.*, 1934, **60**, *Proc.* 52.

16 Spencer, J., D'Amour, F. E., and Gustavson, R. G., *Endocrinol.*, 1932, **16**, 647.

17 Baum, O. S., and Pincus, G., *Am. J. Physiol.*, 1932, **102**, 241.

18 Mahnert, A., *Zentralbl. Gynäkol.*, 1928, **52**, 1754.

19 Dahlberg, G., *Klin. Wchnschr.*, 1930, **9**, 1398.

20 Aron, M., *Arch. d'Anat., d'Histol., d'Embryol.*, 1932, **15**, 237.

8101 P

Persistence of Pituitary Grafts in the Testis of the Mouse.

W. U. GARDNER* AND R. T. HILL. (Introduced by E. Allen.)

From the Department of Anatomy, Yale University.

Heterografts of fresh anterior pituitary tissue have been found to induce both physiological and structural changes, particularly in the gonads. Most implants have been made intramuscularly or subcutaneously. The fate of such implants has either remained unknown or they have been found to undergo necrosis and absorption. Many investigators have found the testis to be a favorable site for ovarian transplants. In some current experiments with mice we have found a very high percentage of successful ovarian grafts in the testis. It was decided to investigate the possibility of anterior lobe tissue becoming more or less permanently established in testicular tissue.

Mice from 22 to 50 days old, and from highly inbred strains, were used.† Littermate brothers or sisters were used as donors. In 12 mice one pituitary was implanted in one testis, and in one animal one pituitary was placed in each testis. The host animals were killed 3 to 12 weeks after implantation. The testes in which grafts had been made were sectioned serially and stained with modified Mallory's triple stain or hematoxylin and eosin. Pituitary tissue was found in the testes of 11 of the 13 mice which were killed. A well-established graft was found in each testis of one animal (1N—Table I). In the 2 unsuccessful cases the hosts' testes had undergone severe necrosis.

Intermediate lobe tissue persisted in most of the grafts, but not as uniformly as anterior lobe tissue. The pars nervosa consistently degenerated. Pars intermedia and pars anterior cells were easily recognizable (Figs. 1 and 2), as well as the normally distinct cleft between the 2 tissues. The anterior lobe tissue was, in all cases, very well vascularized, and appeared normal in arrangement, structure, and types of cells. Although no cell counts were made, the ratio of cells of the various types (acidophils, basophils, and chromophobes) seemed to be entirely normal. The extent of the anterior lobe grafts, as shown by measurements, indicates that most of the implanted tissue had been successfully maintained, as the pitui-

* National Research Council Fellow.

† Mice were very kindly furnished by Dr. L. C. Strong.

FIG. 1.—A photomicrograph of the grafted pituitary tissue recovered from the testis of mouse 5 CBA four weeks after operation. The pars intermedia appears at the upper right. A cleft extends down to the pars anterior cells at the lower border. A mass of necrotic tissue and a large blood vessel lie below the cleft. A normal testicular tubule is observed above the cleft. × 540.

FIG. 2.—A photomicrograph of the grafted pituitary tissue recovered from the testis of a mouse, 7 N, eight weeks after operation. At the upper right were normal appearing pars intermedia cells separated from the pars anterior cells by the normal cleft. Basophilic, acidophilic, and chromophobic cells were present in the well vascularized pars anterior tissue. × 540.

TABLE I.

Mouse No.	Age at grafting days	No. pits grafted	Age of graft days	Donor	Results* Anterior lobe	Results* Interlobe
1N	37	2	21	Brother	+	+
2A	50	1	21	Sister	+	
3A	50	1	21	"	+	+
4A	23	1	28	Brother	+	+
5 CBA	22	1	28	"	+	+
1 CBA	40	1	42	"	+	
8N	40	1	42	"	—	—
7N	40	1	57	"	+	+
10 CBA	27	1	57	"	+	+
9 CBA	27	1	86	"	+	+
3N	40	1	86	"	—	—

* + means good graft; — means no graft.

taries implanted were from immature animals. Mitosis was not observed, indicating that little or no hyperplasia of the persisting tissue was taking place.

Haterius, Schweizer, and Charipper,[1] after making implants of anterior lobe in the anterior eye chamber of rabbits and guinea pigs, observed disturbances of the cell nests and cord arrangement. Also vascular connections were not established. The eye implants show decided contrast to the above described testis grafts in which cell arrangement and ratios appeared quite normal, and in which an excellent host blood supply was established.

Experiments are now in progress to check on the function of anterior pituitary grafts in the testis.

[1] Haterius, Schweizer and Charipper, *Anat. Rec.*, **61**, No. 4.

8102 C

Transperitoneal Absorption. VI. Significance of Impaired Viability and Influence of Distension on its Occurrence.*

LOUIS SPERLING AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

The 3 avenues for absorption of any toxic material from the bowel in simple intestinal obstruction are: the mesenteric veins, the lymphatics and by diffusion through the bowel wall into the peritoneal cavity. It has been shown by many workers that in obstruction, absorption *via* the mesenteric veins is decreased. In another paper we have indicated that lymphatic absorption may be increased by the stasis of obstruction and by increased intra-enteric pressure. We have also indicated in other studies that transperitoneal passage of material probably does not occur unless gross damage to the bowel wall resulting in impairment of its viability is present. Many investigators have emphasized that increased intra-enteric pressure may force toxic material through the bowel wall.

The purpose of this paper is to try to evaluate the occurrence of transperitoneal migration of intestinal content under conditions of obstruction and increased intra-enteric pressure.

Experiments were carried out on: 1. The absorption of strychnine from the normal and obstructed bowel in the intra- and extraperitoneal location. 2. The effect of increased intra-enteric pressure upon the absorption of strychnine from normal and obstructed bowel in the intra- and extra-peritoneal location. 3. The passage of strychnine through the normal devascularized bowel and through devascularized bowel which had previously been subjected to simple ileal obstruction. 4. The effect of increased intra-intestinal pressure upon the passage of strychnine through the normal devascularized bowel wall. 5. The effect of increased intra-enteric pressure upon passage of strychnine through the bowel wall which had previously been subjected to simple ileal obstruction. 6. The effect of increased intra-enteric pressure upon the passage of dyes through the bowel wall.

1. Simple low ileal obstruction was produced in 12 cats. At intervals of 24, 48, 72, and 96 hours of obstruction, 2 cats were operated on again and 10 mg. of strychnine sulphate was injected into the terminal 3 inches of the obstructed bowel, and the bowel

* This work was supported by the Medical Research Fund of the Graduate School, University of Minnesota.

lumen occluded at the site of obstruction by tape ligatures. In one, the loop was returned to the peritoneal cavity and the abdomen closed. In the other, the loop surrounded by warm moist gauze sponges was kept outside the peritoneal cavity, care being taken to avoid compromise of its blood supply. In each experiment the absorption time (interval between injection of strychnine and the onset of convulsive symptoms) was noted. Three normal animals were used as controls to determine the absorption time of strychnine from a normal loop of bowel in both the intra- and extra-peritoneal positions and from the peritoneal cavity directly.

Strychnine was absorbed directly from the peritoneal cavity in 2 minutes. In all of the obstructed animals there was slight but definite delay in increase of absorption time evident within the first 24 hours of obstruction (3 to 6 minutes as compared with 2 to 3 minutes for the normal). This increase of absorption time was more marked when the loops were outside the peritoneal cavity (8 to 13 minutes). In this small series the absorption time seemed to increase directly with the duration of the obstruction.

2. In 4 dogs closed ileal loops 12 inches in length were prepared. A glass cannula was inserted in one end of the loop and after the intra-enteric pressure was raised to 130 mm. of mercury, 50 mg. of strychnine was introduced into the loop. With a pressure maintained over 100 mm. of mercury no absorption could be demonstrated over several hours of observation. Only when the intra-enteric pressure was reduced to approximately 70 mm. of mercury could the strychnine effect be elicited. This occurred whether the loop was in the peritoneal cavity or in an extra-peritoneal location. Two dogs with simple low ileal obstruction of 3 days' duration developed symptoms at 50 and at 60 mm. of mercury when the above experiment was carried out.

When strychnine was introduced into a loop at 100 mm. of mercury and the abdomen closed, convulsions did not develop until the intra-enteric pressure decreased by relaxation of the bowel wall (postural tone) to a level of 75 mm. of mercury at which critical point the strychnine was absorbed *via* the mesenteric vessels.

3. In 4 cats with simple ileal obstruction of 96 hours and one cat with an obstruction of 144 hours' duration, the distal 3 inches of the obstructed gut was devascularized and a large dose of strychnine 10 to 50 mg. was injected into the lumen. Similar devascularized loops were prepared in 5 normal cats as controls. After closure of the abdomen the cats were allowed to come out of anesthesia and observed for signs of absorption of the poison. The obstructed

animals developed convulsive signs within 2½ to 3 hours and were dead or sacrificed within 3 to 4 hours. In contrast, the normal cats did not develop signs of poisoning for 4½ to 7 hours.

4. Closed loops of ileum 10 inches long were prepared in four normal dogs. The loops were devascularized and 50 mg. of strychnine was introduced into the lumen. Two of the loops were distended with air to a pressure above 100 mm. of mercury; the other 2 were not distended. The abdomens were closed. The animals were allowed to come out of anesthesia and were observed carefully for signs of strychnine poisoning. The dogs with the loops under increased pressure developed convulsions within 3 hours. Those in whom the loops were not under pressure remained symptom-free for more than 8 hours.

5. In 3 dogs with simple ileal obstruction of 5 days' duration, closed loops of ileum just above the site of obstruction were made. The intra-enteric pressure was raised to approximately 120 mm. of mercury and 50 mg. of strychnine was introduced into the lumen of the bowel. One dog developed signs of strychnine poisoning in 18 minutes. Examination showed tears of the serosa through to the muscular layers. The second dog developed symptoms in 45 minutes; the third dog did not evidence any symptoms even after 75 minutes. Examination here too revealed numerous tears through the serosa.

6. Freshly excised viable loops of ileum were obtained immediately after death from 2 normal cats sacrificed during the course of other experiments. The loops were filled with 1% trypan blue and subjected to pressures of 50 and 100 mm. of mercury respectively for a period of one hour. Freshly excised loops from 2 cats with low ileal obstruction of 24 and 48 hours' duration were filled with dye and subjected to distension at 40 and 130 mm. of mercury. The dye did not permeate through the bowel wall after one hour of distension. However, when loops from 2 obstructed cats dead 6 and 8 hours were subjected to distension at 100 mm. of mercury, the non-viable bowel wall permitted the dye to permeate directly through it in 15 to 30 minutes. When gentian violet 1% was injected intra-enterally in cats with simple and closed loop ileal obstruction, microscopic section of the bowel wall (Churchman's method[1]) revealed a slight staining of the mucosa in only a few instances. As this was not a constant finding and was present only in the case of cats dead over a period of hours, we must assume that the pene-

[1] Churchman, J. W., *J. Urol.*, 1924, **11**, 1.

tration of the mucosa was a postmortem phenomenon. In none could transperitoneal passage of the dye be demonstrated by wrapping gauze about the outside of the bowel. In one experiment, the dye was introduced into the loop under very great pressure. When the cat was sacrificed 72 hours later (moribund), several purple spots were visible on the antimesenteric border, which were interpreted as representing non-viable necrotic areas through which the dye passed.

Summary. 1. The absorption of strychnine through the mesentery is very rapid even when the loop is outside the peritoneal cavity when contrasted with the absorption time (transperitoneal) when the gut is devascularized. The comparatively slight increase of absorption time in the obstructed loops in the extra-peritoneal position may readily be explained as due to the abnormal location of the loop, slight tension on the mesentery, handling of the bowel, and the accompanying change of temperature which must occur to some extent in spite of attempts to preserve normal conditions. It would appear that obstructed bowel is more sensitive to such changes than the normal.

2. Intra-enteric pressure above the diastolic pressure of the animal (70 mm. of mercury) effectually prevents the mesenteric absorption of strychnine. Such pressures, however, never obtain in experimental or clinical cases of obstruction.

3. From experiments on the devascularized normal and obstructed cat bowel, it appears that the obstructed bowel loses its viability and becomes permeable somewhat sooner than the normal bowel under similar experimental conditions.

4. Devascularized loops of normal bowel permit diffusion of strychnine through the bowel wall in approximately 8 hours and under conditions of increased intra-enteric pressure in approximately 3 hours. Under similar conditions of increased pressure the bowel wall of obstructed dogs becomes permeable somewhat earlier because of tearing of the serosal layers. As shown previously the bowel wall in obstruction is unable to withstand increases of intra-enteric pressure as well as the normal.[2]

5. It is evident that the increased intra-intestinal pressure due to the distension which obtains in simple obstruction is directly responsible for any transperitoneal absorption which might occur. Due to the increased intra-intestinal pressure the bowel wall becomes

[2] Sperling, L., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1138.

damaged, its viability is impaired and diffusion of toxic material through its walls may occur.

6. Experiments on the diffusion of dyes through the bowel wall are in accord with the experiments on strychnine absorption; viable bowel resists permeation and nonviable or dead bowel is readily permeable.

The work of Gatch,[3] Dragstedt,[4] and others concerning the effect of increased intra-intestinal pressure upon the mesenteric absorption is substantiated. The greater potential for the occurrence of transperitoneal absorption in the obstructed bowel is well shown in the results accompanying devascularization of normal and previously obstructed bowel. Still evidence of transperitoneal absorption does not ordinarily obtain until anatomical evidence of bowel wall damage is demonstrable.

Conclusions. 1. Transperitoneal absorption in simple obstruction may occur but only through devitalized segments of bowel subjected to distension. 2. The obstructed bowel under similar experimental conditions is more permeable to strychnine than the normal.

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[3] Gatch, Wm., *et al.*, *West. J. Surg.*, 1932, **40**, 161.

[4] Dobyns, G. J., and Dragstedt, C. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 207.